PATTERNS AND ECOLOGICAL CONSEQUENCES OF ABOVEGROUND AND BELOWGROUND HERBIVORY

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PATTERNS AND ECOLOGICAL CONSEQUENCES OF ABOVEGROUND AND BELOWGROUND HERBIVORY

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Terrestrial plants are embedded in a complex biological network consisting of microorganisms, plants, and animals at several trophic levels. Because plant mass is roughly equally divided above- and belowground, plants function as a key link between the two subsystems. Thus, our understanding of how communities are structured and function may be advanced by considering above- and belowground ecological interactions in tandem.

As a model for understanding above-belowground dynamics, this dissertation focuses on the interactions between common milkweed (*Asclepias syriaca*), a perennial plant native to Eastern North America, and the red milkweed beetle (*Tetraopes tetraophthalmus*), a monophagous herbivore that feeds aboveground as an adult and belowground as a larva. The broad aims of this dissertation are to 1) characterize biotic interactions in the rhizosphere of *A. syriaca*; 2) investigate the individual and community-wide effects of above- and belowground herbivory by *T. tetraophthalmus*; and 3) examine the extent to which *A. syriaca* growth, defense, and ecophysiological traits are genetically correlated in expression, and how these traits affect the performance of *T. tetraophthalmus* adults and larvae.

Soil-dwelling insects commonly co-occur and feed simultaneously on belowground plant parts, yet patterns of damage and consequences for plant and insect

performance remain poorly characterized. **Chapter 1** addresses how two root-feeding insects, *T. tetraophthalmus* and wireworms, affect the performance of *A. syriaca* and the mass and survival of both conspecific and heterospecific insects.

Initial herbivory and induced plant responses have the potential to shape patterns of subsequent insect attack above- and belowground. When different life stages of the same herbivore species damage different plant parts sequentially, there is especially strong potential for induced responses because the sequence of attack is predictable. **Chapter 2** characterizes induced responses of *A. syriaca* following aboveground herbivory by adult, and belowground herbivory by larval, *T. tetraophthalmus* and then tests for changes in the performance of insects that subsequently interact with shoot- and root-damaged plants.

Because many ecological interactions are mediated by plant phenotype, characterizing shoot and root traits is critical for understanding broader scale interactions. **Chapter 3** describes above- and belowground plant traits in terms of heritability, evolvability, pairwise correlations, and clusters and tests for associated impacts on *T. tetraophthalmus* performance.

BIOGRAPHICAL SKETCH

As a child, Alexis often heard the phrase, "After you finish your Ph.D…" from her maternal grandfather, Micheal Evancho. As a teacher and school principal, Dr. Evancho (who earned his doctorate in Vocational Education in 1947) instilled in Alexis a love of learning and appreciation of the value of education. Alexis' mother exemplified these beliefs by earning two masters degrees in Art and Art History, and Library Science and pursuing a doctorate in Instructional Design. Thus, Alexis knew that neither high school nor college would mark the end of her studies. Instead, she expected to pursue graduate work and the question was simply, in what subject?

Alexis' connection to the natural world developed during summers spent as a camper and then a counselor at Camp Catoctin in the Appalachian Mountains of Maryland. Like so many who become ecologists, she was enthralled with biodiversity, scenic landscapes, and phenological patterns in nature. This sentiment encouraged her to pursue an internship in a laboratory during the spring of her senior year of high school. She was lucky to be welcomed into the lab of Dr. Roger Hanlon at the Marine Biological Laboratory in Woods Hole, MA. There she gained first-hand knowledge of the scientific process by independently developing experimental materials and working with graduate students to conduct behavioral trials and analyze data. Alexis' contributions to the project, which characterized the chemosensory cues male squid use to gain access to egg bundles, resulted in her first paper, published in the *Journal of Chemical Ecology* not long after she graduate from high school.

Alexis continued to study science at Brown University, where she earned a B.S. with honors in Geological Sciences-Biology. A seminal experience in her

academic career occurred during her year abroad at the University of Otago in New Zealand. She realized that she wasn't cut out to be a geologist when, at a rock outcrop, she noticed that her classmates were most interested in the formation while she was interested in the plants growing on top.

Alexis returned to Brown and, in consultation with her insightful and supportive advisor, Dr. Jan Tullis, decided to pursue an Honors Thesis in the Department of Ecology and Evolutionary Biology, rather than in Geological Sciences. Alexis was confidant in her decision to switch when Dr. Tullis noted that geology was the "study of experiments that Nature has already run" whereas biological sciences offers the experimenter more control.

Alexis had the honor and great pleasure to work with Dr. Doug Morse for the next two summers at his field site near Bremen, Maine. Together, they investigated diurnal and seasonal patterns of nectar secretion in common milkweed and the ragged fringed orchid, two plant species that naturally co-occur in old-fields, but have strikingly different pollination ecology. Dr. Morse and Dr. Jon Whitman, both caring mentors, encouraged Alexis to apply to Cornell for a doctorate. The opportunity to work with Dr. Anurag Agrawal at Cornell was quite exciting, as he studied the same system (common milkweed) from a different perspective (herbivory, rather than pollination). Supported by the tremendous human and material resources available at Cornell and in the Agrawal lab, Alexis researched the patterns and ecological consequences of aboveground and belowground herbivory on common milkweed. As a postdoctoral fellow at the California Council on Science and Technology, Alexis will serve the state legislature by recommending solutions to science and technology-

related policy issues.

To Christine Broening, Jan Tullis, and Doug Morse,

for their many kinds of support.

To John Erwin, who once said, “Our armies of PhDs – hard-working deluxe technicians with their suppressed imaginations – are disarmed when their specializations become obsolete. Aardvark.”

and

To Stephanie Evancho, a fearless healer

who guided me on the path of understanding and love.

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I also would like to thank my Special Committee members, Professors Monica Geber and Jed Sparks. As acting advisor while Anurag was on sabbatical and as Director of Graduate Studies, Monica has played a critical role in my professional and personal development. I am grateful for her many, diverse forms of support. I appreciate Jed for improving my experimental designs, helping me to think about plant biology in broader and sophisticated ways, and offering welcome advice on life in and outside of graduate school.

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CHAPTER ONE

SPECIFIC IMPACTS OF TWO ROOT HERBIVORES AND SOIL NUTRIENTS ON PLANT PERFORMANCE AND INSECT-INSECT INTERACTIONS

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ABSTRACT

Soil-dwelling insects commonly co-occur and feed simultaneously on belowground plant parts, yet patterns of damage and consequences for plant and insect performance remain poorly characterized. We tested how two species of root-feeding insects affect the performance of a perennial plant and the mass and survival of both conspecific and heterospecific insects. Because root damage is expected to impair roots' ability to take up nutrients, we also evaluated how soil fertility alters belowground plant-insect and insect-insect interactions. Specifically, we grew common milkweed (*Asclepias syriaca*) in low or high nutrient soil and added seven densities of milkweed beetles (*Tetraopes tetraophthalmus*), wireworms (mainly *Hypnoides abbreviatus*), or both species. The location and severity of root damage was species-specific: *Tetraopes* caused 59% more damage to main roots than wireworms, and wireworms caused almost seven times more damage to fine roots than *Tetraopes*. *Tetraopes* damage decreased shoot, main root, and fine root biomass, however substantial damage by wireworms did not decrease any component of plant biomass. With the addition of soil nutrients, main root biomass increased three times more, and fine root biomass increased five times more when wireworms were present than when *Tetraopes* were present. We detected an interactive effect of insect identity and nutrient availability on insect mass. Under high nutrients, wireworm mass decreased 19% overall and was unaffected by the presence of *Tetraopes*. In contrast, *Tetraopes* mass increased 114% overall and was significantly higher when wireworms were also present. Survival of wireworms decreased in the presence of *Tetraopes*, and both species' survival was negatively correlated with conspecific density. We conclude that insect identity, density, and soil nutrients are

important in mediating the patterns and consequences of root damage, and suggest that these factors may account for some of the contradictory plant responses to belowground herbivory reported in the literature.

INTRODUCTION

Decades of research have yielded a broad understanding of interactions between plant shoots and aboveground insect herbivores. In contrast, our understanding of belowground herbivory remains limited, despite calls for greater attention to the impacts of root-feeding insects (Brown and Gange 1990, Blossey and Hunt-Joshi 2003, Rasmann and Agrawal 2008, Erb et al. 2012) and evidence that the effects of belowground herbivores are fundamentally different, and sometimes more detrimental, than the effects of aboveground herbivores (reviewed in Brown and Gange 1990, Zvereva and Kozlov 2011). Because belowground plant parts—including rhizomes, storage organs, vascular roots, and root buds—constitute 50-90% of total plant biomass (Andersen 1987, Bazzaz et al. 1987), belowground herbivory can have important effects that extend well beyond the scope of tissue damage (reviewed in Zvereva and Kozlov 2011, Johnson et al. 2012). These include effects on individual plant chemistry, biomass, and survival (e.g., Reichman and Smith 1991, Maron 1998, Rasmann et al. 2009, Coverdale et al. 2012), interactions with other trophic levels both above and belowground (e.g., Wäckers and Bezemer 2002, Rasmann et al. 2011), and plant distribution and community structure (e.g., Brown and Gange 1989, Stein et al. 2010, Coverdale et al. 2012).

A recent meta-analysis confirmed that belowground herbivory causes negative impacts on root biomass and associated changes in plant performance; however, the

magnitude of these effects depend on the plant part, morphological trait, and function in question (Zvereva and Kozlov 2011). The impacts of belowground herbivory on plant performance have also been shown to vary with plant nutritional status (Brown and Gange 1990, Johnson et al. 2009), plant defenses (Bezemer and van Dam 2005, Rasmann and Agrawal 2008, van Dam 2009), root feeder identity (Müller 1989, Steinger and Müller-Schärer 1992, Wurst and Van der Putten 2007), and root feeder density (Masters 1995, Strong et al. 1995, Onstad et al. 2006, Simelane 2010). Despite these foundational studies, questions about context-dependency in belowground plant-insect interactions remain.

Here, we report on an experiment using the perennial plant common milkweed (*Asclepias syriaca*, Asclepidaceae) and two types of root-feeding insects that naturally co-occur within its root system (A. Erwin, *personal observations*): generalist wireworms (Elateridae) and specialist red milkweed beetles (*Tetraopes tetraophthalmus*, Cerambycidae). By adding these insects at seven densities to plants growing in either low or high nutrient soil, we were able to ask the following questions: (i) What is the location and extent of root damage caused by each insect, and are these patterns influenced by insect density and soil nutrients? (ii) How does damage influence plant root and shoot biomass? (iii) How do conspecific and heterospecific interactions between insects affect their individual mass and survival, and are these interactions altered by soil nutrients?

In a previous study (A. Erwin, *unpublished data*), we grew common milkweed from seed, transplanted seedlings to the field, and added five red milkweed beetle larvae to half of the plants. During the harvest of this experiment, we observed that wireworms had naturally recruited to many plants' root systems (A. Erwin, *personal observations*).

Although we did not test for whether wireworm abundance depended on the presence of *T. tetraophthalmus* larvae, their natural co-occurrence motivated us to investigate the ecology of these root-feeders in the milkweed rhizosphere.

In the present study, we predicted that (i) wireworms would predominantly damage fine roots, given their putatively higher nitrogen concentration (Gordon and Jackson 2011) and lower defenses (Griffith 1974, van Dam 2009); (ii) *T. tetraophthalmus* would predominantly damage main roots, based on previous observations of their boring habit (Williams 1941, Chemsak 1963, Rasmann et al. 2011); (iii) high soil nutrients would increase the plants' ability to compensate for damage, and this effect would vary by plant part and insect species. Finally, we predicted that (iv) the performance of the generalist, omnivorous wireworms would decrease with intraspecific competition and increase with the presence of *T. tetraophthalmus*, a potential prey species. In contrast, we expected that performance of the specialist herbivore *T. tetraophthalmus* would not be negatively density-dependent (because eggs are laid in clutches and so larvae may be adapted to intraspecific competition), but would decrease in the presence of wireworms, which are potential predators. Despite expectations that insect identity, density, and soil nutrients are major factors shaping patterns of belowground herbivory, we currently have a limited understanding of their independent and interactive effects (Zvereva and Kozlov 2011).

MATERIALS AND METHODS

Study System. Common milkweed *Asclepias syriaca* L. is a perennial herb found in disturbed areas and early successional habitats across eastern North America (Wyatt

1996). In central New York, USA, ramets emerge in late May and flower from mid-June through July. Although ramets can produce multiple fruits that each contain up to ~200 seeds, reproduction is primarily asexual and occurs belowground via the production of new ramets (Woodson 1954). The root system of *A. syriaca* seedlings consists of a single 'main' root (mean diam. 4.5 ± 1.1 mm, usually third order) as well as numerous very 'fine' roots (mean diam. 0.5 ± 0.25 mm, first and second order) (A. Erwin, *unpublished data*). From visual inspection and in comparison to other species (Pregitzer et al. 2002, Guo et al. 2008), it is clear that the fine root system of young common milkweed plants is dominated by few, long, relatively unbranched individual roots rather than an intricately branched system of many short roots (A. Erwin, *personal observations*). Other belowground structures exist within milkweed root systems—including adventitious root buds and, in mature field plants, lateral branch roots and a rhizome—but these were not considered in the present study.

The red milkweed beetle *Tetraopes tetraophthalmus* Forster is a monophagous herbivore of *A. syriaca*. Adults emerge from the soil in late June and begin to feed on milkweed flowers and foliage (Matter 2001). Females oviposit clutches (10-15 eggs each) in dry stems of nearby grasses and forbs (Gardiner 1961, Agrawal 2004). Larvae hatch after approximately 6-10 days, drop to the ground, and begin to feed on milkweed roots and rhizomes (Agrawal 2004). Larvae overwinter in earthen cells as prepupae (Williams 1941, Chemsak 1963).

Wireworms (the juvenile stage of click beetles) are a species group of generalist omnivores that feeds on soil-dwelling insects, soil organic matter as well as the belowground parts of living plants from several botanical families, including weeds and

crops (Griffith 1974). Wireworm neonates fail to survive in soils that do not contain plant roots or storage organs (Rawlins, 1940). Second- and third- instar larvae are fairly tolerant of starvation, however growth slows and mortality is high when no belowground plant material is available for food (Rawlins, 1940). Unlike univoltine *T. tetraophthalmus*, wireworms persist in the soil as larvae for 2-7 years, moving up and down in the soil profile as food availability, temperature, and soil moisture change (Penev 1992, Benefer et al. 2012). Adults emerge in late spring and remain active aboveground throughout the summer. A female adult may lay from 50 to 300 eggs, singly or in small clutches, 2.5 – 15 cm deep in the soil of grassy fields (Andrews et al. 2008, Kuhar and Alvarez 2008). An early survey of agricultural fields demonstrated that average abundance increases from 60,000 wireworms per acre in 1-3 year old grasslands to 230,000 wireworms per acre in grasslands older than 10 years (Fox 1961). Because of their long life cycle, larvae may persist in former agricultural areas (old fields), where common milkweed is typically found.

Plant and insect collection. In September 2009, we collected *A. syriaca* seeds from 40 discrete patches around Ithaca, NY, USA. In May 2010, we nicked each seed coat with a sterile blade, stratified the seeds on moist filter paper at 4 °C for 1 week, and germinated them in a dark chamber at 28 °C for several days (Rasmann et al. 2009).

Tetraopes tetraophthalmus adults were collected in June 2010 on naturally occurring *A. syriaca* patches around Tompkins County, NY, USA and kept in ventilated rearing boxes (30 × 20 × 15 cm) in the laboratory. Males and females were provided with fresh milkweed leaves and flowers for food, and dry grass stems as oviposition substrate (Rasmann et al. 2009). The substrate was removed from the rearing containers every third

day and incubated in the dark at 27 °C for 1 week (Rasmann et al. 2011). Newly hatched larvae were kept without food in petri dishes (10 cm diam.) on moist filter paper for 24 hours before being added to the roots of experimental plants (Rasmann et al. 2011). When added, individual *T. tetraophthalmus* fresh mass was on average 0.133 ± 0.005 mg (n = 20).

Wireworms were collected in June 2010 from soil below recently overturned sod in Steuben County, NY, USA. Larvae were kept at room temperature in ventilated containers (30 × 20 × 15 cm) filled with local field soil and placed in a dark area of the laboratory. Every third day, we moistened the soil and provided larvae with fresh potato pieces for food. Larvae were kept in this condition without food for 24 hours before being added to experimental plants. When added, individual larvae had a mean fresh mass of 17.574 ± 1.440 mg (n = 25). A subsample of the wireworms used in our experiments was identified at the Cornell University Insect Diagnostic Laboratory, indicating that our collection was comprised of two generalist omnivores: *Hypnoides abbreviatus* (83%) and *Agriotes mancus* (17%).

Experimental set-up and data collection. Because of the relatively small spatial scale (< 1 m^3) of rhizosphere dynamics (Van der Putten et al. 2001, Phillips et al. 2009), root-insect interactions can be studied semi-realistically in mesocosms. Here, we used plastic pots in a growth chamber. We lined each pot (10 cm diam.) with a layer of nutrient- and water-permeable fabric (Harvest Guard, Dalen Products, Knoxville, TN, USA) to discourage insects from exiting via pot drainage holes. We filled the pots with an equal volume of a 1:2 mixture of sand and a growing mix recommended for seed germination that included Sphagnum peat moss, perlite, gypsum, and dolomitic

limestone, but no nutrient charge (Sunshine Mix #2, Sungrow Horticulture, Bellevue, WA, USA). We incorporated sand to improve pot drainage and facilitate fine root recovery. We individually transplanted seeds into pots in a growth chamber (14:10 hours D/N light, and 26:16 °C temperature) and watered *ad libitum* every other day. The seedlings grew slowly because micronutrients were not present in the soil mix. After two weeks, we completely randomized the plants and initiated the nutrient treatments. Plants in the high nutrient treatment received inorganic fertilizer [N:P:K 21:5:20 150 ppm N (g/g), Table S1.7] once every week and plants in the low nutrient treatment received the same fertilizer once every other week. We chose these treatments based on a pilot study in which seedlings were grown under one of six fertilizer treatments that span the range of soil fertility found locally. After four weeks, stem height and leaf number differed between the two nutrient levels that we selected for the present study (stem height low: 17.80 ± 4.01 cm, high: 24.61 ± 4.97 cm; leaf number low: 5.87 ± 0.73, high: 8.41 ± 1.69).

After initiating the nutrient treatments, we allowed the plants to grow for an additional four weeks. At the six-week mark, plants received one of three insect treatments: i) *T. tetraophthalmus*, ii) wireworms, or iii) both. The insect treatments were divided by density level such that plants with either single-insect treatment received 0, 1, 2, 3, 4, 5 or 6 larvae, and plants with the dual-insect treatment received 0, 1, 2, or 3 larvae of each insect (and thus had 0, 2, 4, or 6 total larvae). We chose to standardize insect treatments according to density, acknowledging that this did not result in treatments with comparable mass. Densities fall within the range we observed in 2010 in a local field (A. Erwin, *personal observations*). With seven replicates per density treatment combination, this design yielded N = 240 plants. To apply the insect(s), we made a small hole (2.5 cm

away from the plant stem, 1 cm deep) in the soil mixture with the end of a paintbrush, into which we gently placed the larva(e). Plants and insects were left to grow for an additional four weeks.

At harvest, we carefully loosened the soil mixture and collected surviving insects by hand. Insects were brushed to remove soil, counted, and weighed. Because initial weight was very consistent within a species (see Methods) we did not weigh each individual larva before adding it to an experimental pot. Consequently, instead of calculating insect growth as final weight minus initial weight, we calculated growth on a per pot basis as the total mass of surviving larvae divided by the number of surviving larvae. This metric allows us to test for any effects of our treatments on mass without potentially confounding mass with survival. We calculated insect survival as the number of surviving larvae of a given species re-collected from a pot relative to the number that we added initially.

We then washed the roots and separated the main root from the fine roots using a sterile blade. We quantified main root length with a ruler. Damage to the main root (Fig. 1.1A) was visible to the unaided eye and characterized by direct consumption in the form of bore marks, i.e., 'tunnels' through an outer 'shell' of main root. Damage to all fine roots (Fig. 1.1B) was also readily apparent and characterized by the presence of black

necrotic tissue at the site of feeding. The ends of some fine roots were probably consumed (as suggested by flat, rather than pointed, fine root tips), however the lengths of these consumed tissues were impossible to quantify because the tip had been removed and an outer 'shell' was not present. To the extent that this type of consumption occurred, our index of fine root damage is conservative. Our approach to quantifying damage—

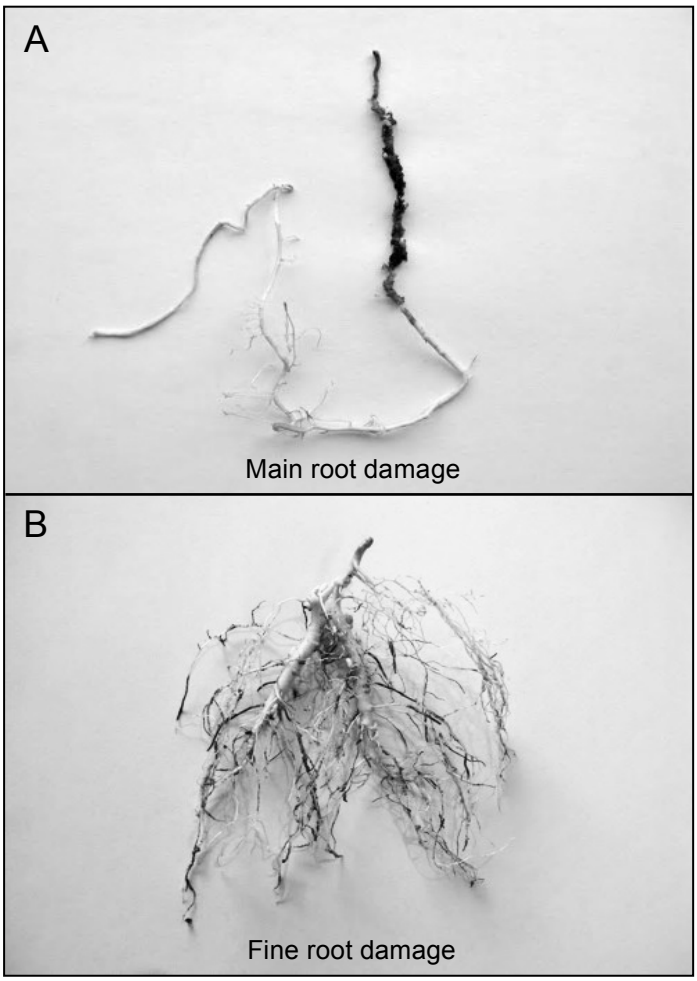

Figure 1.1 A) A washed *Asclepias syriaca* root system in which three *T. tetraophthalmus* larvae had been feeding for four weeks. Fine roots, which had no herbivore damage, were removed to better show damage to the main root. Damage to the main root was measured as the length of the single section of black necrosis. B) A washed *Asclepias syriaca* root system in which three wireworm larvae had been feeding for four weeks. Damage to fine roots was measured as the sum of the length of all necrotic sections (n = 43).

measuring individual sections of damaged tissue and summing over the entire root system—is considerably more detailed than other methods that are qualitative or based on a visual scale (Spike and Tollefson 1988, Agrawal 2004, Simelane 2010, Rasmann and Agrawal 2011, but see Oleson et al. 2005).

Since we did not recover a main root from some plants that received 4, 5 or 6 larvae, we did not measure damage to this root type. However, we did recover 'orphaned' fine roots (those no longer attached to the main root, but not yet decomposed) from these plants. Therefore, we are confident that the main root was absent due to consumption, rather than decomposition, because fine roots generally have higher decomposition rates than larger diameter roots. Therefore, we calculated the mean main root length for each treatment and substituted these values (Table S1.1) where data were missing. We assumed that main root length equaled main root damage because the entire main root was consumed. We performed analyses with and without the substituted data and found that the direction and magnitude of the overall patterns and all statistical results remained unchanged. After quantifying root damage, all root and shoot material was oven-dried at 40 °C for 3 days and weighed.

Statistical analyses. We performed analysis of covariance (ANCOVA) in JMP (ver. 8. SAS Institute Inc., Cary, NC) to test for the fixed main effects of insect identity (discrete, factor), insect density (continuous, co-factor), and soil nutrient level (discrete, factor) and their interactions on damage to main and fine roots. Because we were interested in examining the full range of insect density (i.e., 1, 2, 3, 4, 5, 6), we excluded data from the dual-insect treatment because it lacked data at density levels 1, 3, and 5. We excluded the controls (density 0) because no damage was observed on their roots. We

employed the same model to test for the effects on shoot, main root, and fine root biomass except that, here, we included the controls to determine potential change from an undamaged baseline. Data were log-transformed prior to the biomass analysis to meet assumptions of normality and homogeneity of variance.

To analyze insect mass, we separated the overall dataset (including all density levels except controls) into two subsets: (i) wireworm mass when alone and in the presence of *T. tetraophthalmus*, and (ii) *T. tetraophthalmus* mass when alone and in the presence of wireworms. For each subset, we performed ANCOVA to determine how insect mass was affected by insect identity, insect density, soil nutrient level, and their interactions.

To analyze insect survival, we again separated the overall dataset into two subsets. Based on previous work in this system (Agrawal 2004, Rasmann et al. 2009, 2011), we generated *a priori* a set of nine candidate models that represent survival as a function of the main effects and/or interaction terms. We used generalized linear modeling to fit our data to these models according to a binomial error distribution with logit as link function (Quinn and Keough 2002). Our data conformed to variance assumptions based on binomial distributions (wireworm data subset: $c = 1.07$; *T. tetraophthalmus* data subset: $c = 0.99$; (Burnham and Anderson 2002). We evaluated the fit of each candidate model using the Akaike's Information Criterion corrected for small sample sizes, AICc (Akaike 1973), and associated statistics: delta AICc (Δ_i), Akaike weight (w_i), and log likelihood values with standard cutoffs (Burnham and Anderson 2002). We also calculated model-averaged coefficients and unconditional confidence intervals (CI) based on Akaike weights for each parameter. Finally, we calculated relative

importance (RI) values to estimate the relative importance of each parameter in each data subset. RI values are calculated as the sum of all weights from models that include that parameter; the higher the RI value, the more important that parameter is relative to others (Burnham and Anderson 2002). Survival analyses were conducted in R ver. 2.13.0, The R Foundation for Statistical Computing.

Note that in our analyses of plant traits, the insect identity term refers to the difference between the effects of wireworms and *T. tetraophthalmus* and so a significant identity-by-density interaction would indicate that the effect of increasing density on plant damage or biomass depended on *which insect* was present. In contrast, in our analyses of insect traits, the insect identity term refers to the presence of the other insect on the performance of the focal insect. In these cases, a significant identity-by-density interaction would indicate that the effect of increasing density on a focal insect's mass or survival depended on *whether heterospecifics* were present, e.g., wireworms alone vs. wireworms in the presence of *T. tetraophthalmus*.

RESULTS

The damage to *A. syriaca* main roots (Fig. 1.1A) depended on insect identity and density (Table 1.1). Averaged across all density and nutrient levels (but not including undamaged controls), *Tetraopes tetraophthalmus* damaged 6.31 ± 0.63 cm of each plant's main root, which is 59% more damage than wireworms caused (Table S1.2). Damage to main roots was positively correlated with overall insect density, and increased with increasing density of *T. tetraophthalmus*, but not wireworms (Fig. 1.2). Under high soil nutrients, mean damage to main roots by *T. tetraophthalmus* increased by 44% and by

wireworms by 2% (Table S1.2), but neither the effect of nutrients nor the insect identity-by-nutrients interaction were significant (Table 1.1). Damage to main roots increased with insect density under high, but not low soil nutrients, as shown by a significant density-by-nutrients interaction (Table 1.1).

Like damage to main roots, damage to fine roots (Fig. 1.1B) was affected by insect identity and density (Table 1.1). Averaged across all density and nutrient levels (excluding controls), wireworms damaged 3.75 ± 0.43 cm of each plant's fine roots, which is almost seven times more damage than *T. tetraophthalmus* caused (Table S1.2), and this difference was especially large at high insect densities (Fig. 1.2). Damage to fine roots was 54% higher under high nutrients overall (Table S1.2), but neither the effect of nutrients nor the interactions of other factors with nutrients were significant (Table 1.1).

Insect identity and density also influenced final above and belowground plant biomass (Table 1.1). Averaged across all density and nutrient levels (including controls), mean main root biomass was 180% lower when *T. tetraophthalmus* were present than when wireworms were present (Table S1.2). This result is consistent with the insect-specific patterns of main root damage, noted above. *Tetraopes tetraophthalmus* caused more modest, but still highly significant decreases in mean fine root and shoot biomass (36% and 15%, respectively) compared to wireworms (Tables 1.1, S1.2). All components of plant biomass were affected by insect density (Table 1.1), but reductions in biomass were most pronounced in fine roots and especially main roots exposed to *T. tetraophthalmus* (Fig. 1.3). Indeed, when we excluded the *T. tetraophthalmus* data and again tested for the effects of density, nutrients, and their interaction on plant biomass, we found that only nutrients remained significant (Table S1.4). This result indicates that

	Plant Damage		Plant Biomass			Insect Mass	
Factor	MR (1, 145)	FR (1, 151)	SH (1, 178)	MR (1, 179)	FR (1, 179)	Wireworms (1, 63)	*T. tetraophthalmus* (1, 37)
Identity	**7.14****	**28.18*****	**35.43*****	**276.31*****	**74.00*****	0.29	0.77
Density	**10.35****	**19.55*****	**5.93***	**57.58*****	**21.91*****	**10.04****	0.88
Nutrients	1.80	2.15	**130.27*****	**6.46***	**16.45*****	**4.93***	**12.05****
Identity*Density	**8.80****	**18.97*****	1.54	**56.93*****	**11.40*****	0.44	0.51
Identity*Nutrients	1.54	1.07	2.25	**4.15***	**8.20*****	1.07	**7.22***
Density*Nutrients	**3.97***	1.14	1.60	0.74	0.12	2.07	0.47
Identity*Density*Nutrients	0.12	1.57	0.64	0.50	1.03	0.85	0.56

Table 1.1 Analysis of covariance (ANCOVA) of insect identity, insect density, soil nutrient level and their interactions as predictors of *Asclepias syriaca* main root (MR) and fine root (FR) damage (cm), shoot (SH), MR, and FR biomass (mg), and mass of wireworm and *Tetraopes tetraophthalmus* larvae (mg). Numbers in parentheses below column headers refer to df. Numbers in table are F-values with asterisks indicating significant differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). For plant damage and biomass, the Identity term refers to which species was present. For insect mass, however, the Identity term refers to whether heterospecifics were present.

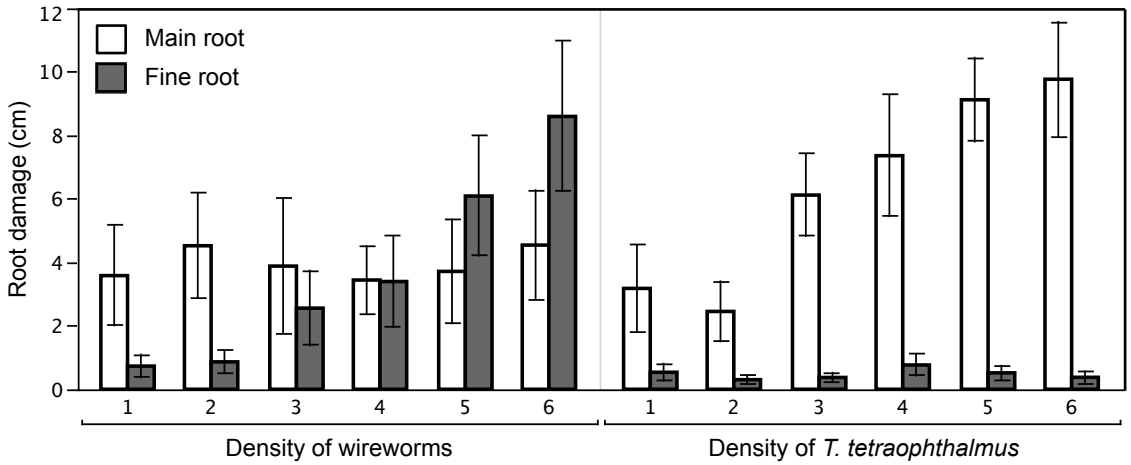

Figure 1.2 Mean ± standard error damage to the main and fine roots of *Asclepias syriaca* fed upon by different densities (1 to 6 larvae per plant) of wireworms or *Tetraopes tetraophthalmus.* Control (0 larvae) plants were excluded because no damage was observed on their roots.

the significant effects of density, identity-by-density, and identity-by-nutrients in the full model (Table 1.1) are driven by the overwhelming influence of *T. tetraophthalmus*.

The biomass of all plant parts was influenced by soil nutrient level (Table 1.1), but increases were more pronounced in shoots (a 30% mean increase overall) than in either root type (a 16% mean increase in both main and fine roots overall, Table S1.2). Soil nutrient level affected aboveground plant biomass, regardless of which insect was added (Table 1.1). In plants without insects (i.e., controls) and plants exposed to wireworms or *T. tetraophthalmus*, shoot biomass increased 39%, 33% and 29%, respectively (Fig. S1.1A). However, the pattern for belowground biomass was insect-specific, as indicated by a significant identity-by-nutrients interaction (Table 1.1). In plants exposed to wireworms, the mean biomass of main roots and fine roots increased under high nutrients by 20% and 26% respectively (Fig. S1.1B, C), whereas in plants exposed to *T. tetraophthalmus*, increases in mean main root and fine root biomass were much smaller (5% and 4%, respectively) and not significant (Fig. S1.1B, C). In plants without insects, we did not detect significant increases in belowground biomass insect density influenced the mass of individual wireworms, but not the mass of individual *T. tetraophthalmus* (Table 1.1). Soil nutrient level influenced the mass of both insects (Table 1.1), but in opposite directions: wireworm mass decreased by 23% under high nutrients whereas *T. tetraophthalmus* mass increased by 114% (Table S1.3). This result is consistent with the result that *T. tetraophthalmus* has greater impacts on belowground biomass than wireworms, especially under high nutrients. We did not detect a significant interaction between the presence of heterospecifics and soil nutrients for wireworm mass (Table 1.1, Fig. 1.4A). In contrast, we did detect an identity-by-

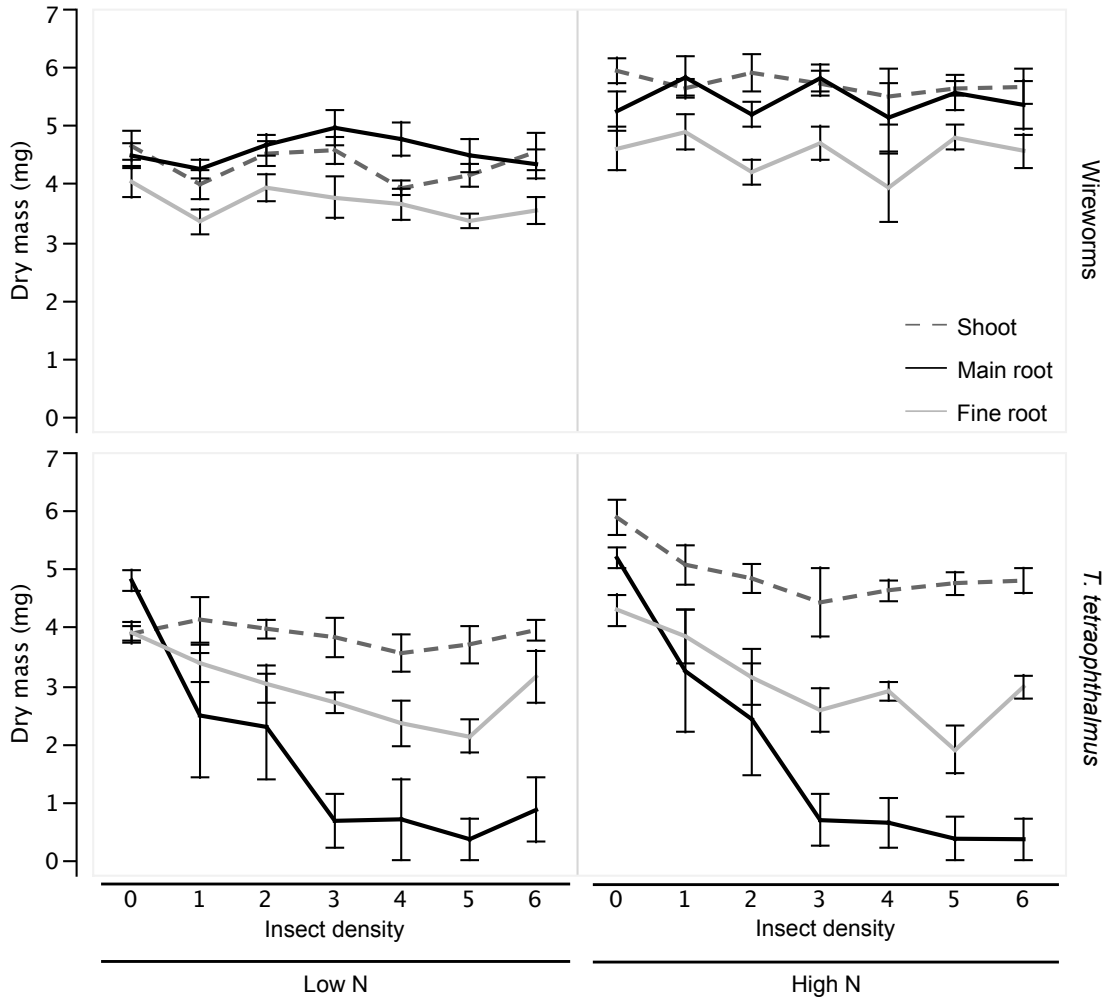

Figure 1.3 Mean ± standard error shoot, main root, and fine root dry mass of *Asclepias syriaca* plants fed upon by different densities of wireworms or *Tetraopes tetraophthalmus* larvae and grown in either low or high nutrient soil.

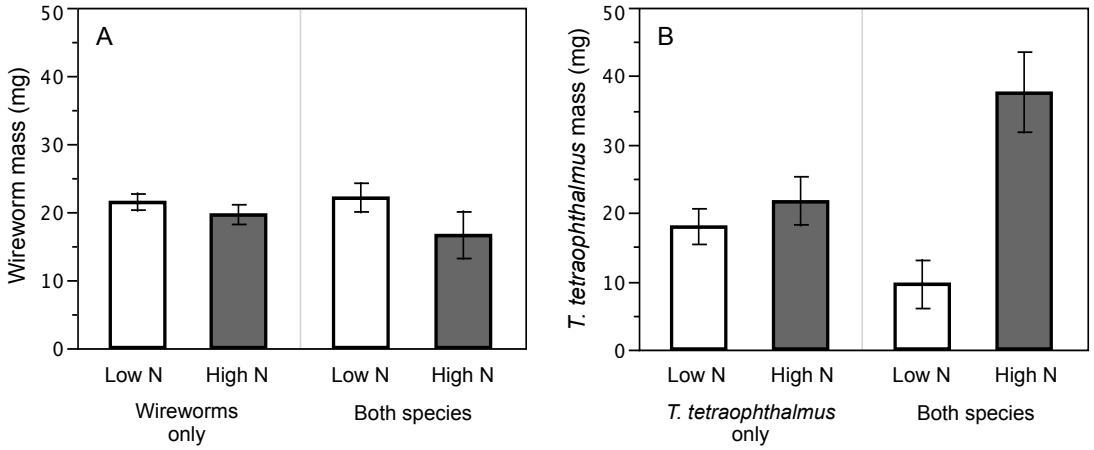

Figure 1.4 Mean ± standard error mass of individual (A) wireworms and (B) *Tetraopes tetraophthalmus* larvae when co-occurring with conspecifics only or conspecifics plus heterospecifics under low or high nutrient soil. Total insect density was controlled in all comparisons.

nutrients interaction for *T. tetraophthalmus* (Table 1.1), whose mass increased by 20% under high nutrients when it occurred alone, and by almost 3-fold when it occurred with wireworms (Table S1.3, Fig. 1.4B).

The best model for predicting wireworm survival included insect identity (indicating the presence of heterospecifics) and conspecific insect density ($AICc_{best}$: 289.15, Table S1.5). The confidence intervals around these two coefficients did not overlap zero (CI_I: 0.09, 1.62; CI_D: -0.35, -0.01, Table S6). In addition, the relative importance values for insect identity and density were high (0.89 and 1.0, respectively, Table S1.6), lending further support to the inference that wireworm survival decreased when it occurred with *T. tetraophthalmus* and with increasing conspecific density (Fig. 1.5A). The impact of conspecific density on wireworm survival did not appear to depend on the presence of *T. tetraophthalmus* because the identity-by-density interaction did not appear in the best model.

The best model for predicting *T. tetraophthalmus* survival included insect density only ($AICc_{best}$: 172.98, Table S5). The CI around the density coefficient excluded zero (CI_D: -0.73, -0.17, Table S6). Moreover, the relative importance of density for predicting *T. tetraophthalmus* survival was 1.00, showing very strong support for the inference that *T. tetraophthalmus* survival was influenced negatively by conspecific density (Table S1.6, Fig. 1.5B).

DISCUSSION

A continuing challenge in ecology is to predict the specificity and abiotic context-dependency of species interactions (Agrawal et al. 2007). We tested how

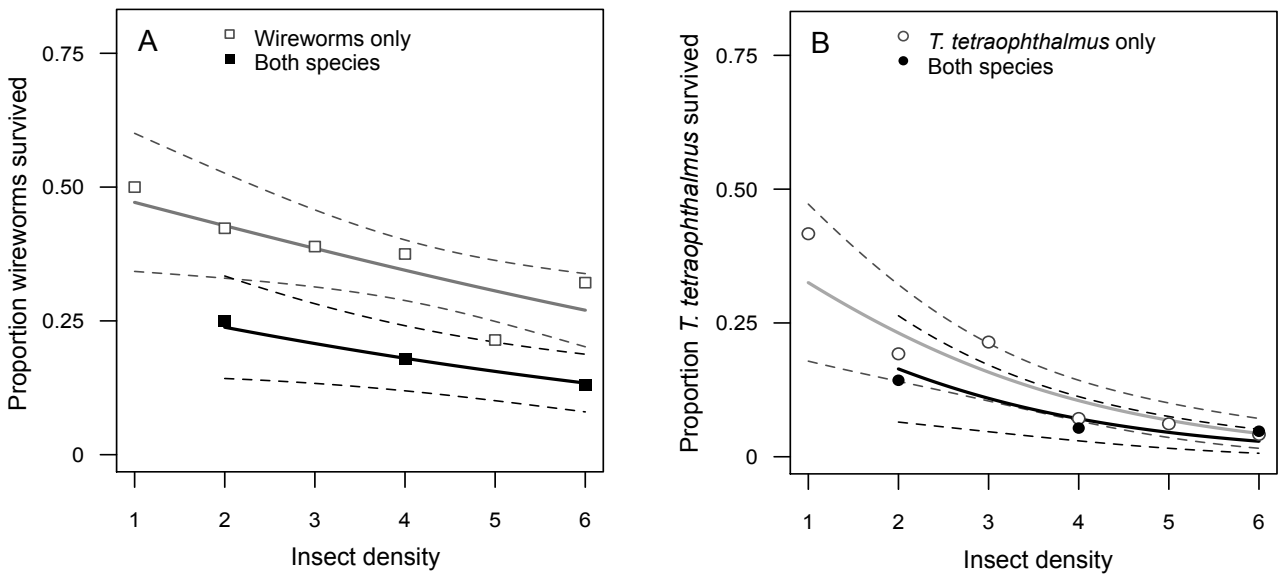

Figure 1.5 Proportion of insects that survived when co-occurring with conspecifics only or conspecifics plus heterospecifics. Total insect density was controlled in all comparisons. Solid lines indicate the predicted proportion insect survival based on logistic regression for plants to which a single species was added (grey lines) or both species were added (black lines). Dashed lines indicate 95% confidence intervals for each predicted fit. Symbols indicate the mean proportion survival at a given density level.

belowground plant-insect and insect-insect interactions were influenced by insect identity, insect density, and soil nutrients. We showed that two types of root-feeding beetles differed in the location and severity of damage they imposed on common milkweed roots and in the consequences of that damage for plant root biomass and the mass and survival of individual insects. Additionally, we demonstrated that insect density and soil nutrients play a role in mediating root-herbivore interactions: the density of conspecifics was negatively correlated with the survival of both insects, but the presence of heterospecifics was important for only one insect; nutrients affected plant and insect mass, but not root damage. These results add to the growing appreciation of belowground herbivory as an inconspicuous yet critically important factor shaping plant and insect performance.

Species-specific impacts of belowground herbivory. We detected specific effects of root damage on below- and aboveground plant biomass. Considerable damage—mostly to fine roots, as predicted—by high densities of wireworms did not reduce belowground biomass, indicating that compensatory growth occurred. Plant compensation for belowground damage (imposed by herbivores or mechanically) has been previously reported, especially in agricultural systems (reviewed in Andersen 1987, Brown and Gange 1990) and there is also widespread evidence of compensation and overcompensation to aboveground herbivory (reviewed in Trumble et al. 1993). In contrast to damage by wireworms, extensive damage by *T. tetraophthalmus*—almost exclusively to main roots, as predicted—decreased the biomass of both main roots and fine roots, indicating little to no compensation as well as 'downstream' effects on fine

root biomass. Although neither insect interacted directly with plant shoots, root damage by *T. tetraophthalmus* reduced root and, in turn, shoot biomass, as has been reported in other systems (Morón-Ríos et al. 1997, Wardle et al. 2004). In contrast, root damage by wireworms reduced neither root nor shoot biomass.

Species-specific impacts of root-feeding insects on plant performance have been reported in other systems (reviewed in Andersen 1987, Brown and Gange 1990, Blossey and Hunt-Joshi 2003). For example, Sheppard et al. (1995) showed that when *Carduus nutans* (Asteraceae) plants were exposed to root-feeding larvae of *Hadroplontus trimaculatus* (Curculionidae) and *Cheilosia corydon* (Syrphidae), damage by the weevil resulted in more stems being produced whereas damage by the syrphid fly reduced seed production. More recently, Wurst and Van der Putten (2007) demonstrated that wireworms (*Agriotes* spp., Elateridae) decreased total root biomass and led to compensatory shoot growth in *Plantago lanceolata* (Plantaginaceae), however plant parasitic nematodes had no effect on root or shoot biomass.

We suggest that the location of root damage is a key factor in explaining specific impacts of belowground herbivory on root and shoot biomass. Compensation may be more common when belowground damage is concentrated on fine roots, which are generally less costly to produce, less well defended, and often have a high turnover rate (van Dam 2009, Hodge et al. 2009, Eissenstat and Yanai 1997). In addition, main roots, if undamaged, can potentially mobilize stored resources to maintain fine root and shoot biomass (Trumble et al. 1993). Steinger and Müller-Schärer (1992) showed that specific impacts of two root-feeding insects (the moth *Agapeta zoegana* Tortricidae and the weevil *Cyphocleonus achates* Curculionidae) on host plant (*Centaurea maculosa*

Asteraceae) biomass were produced not by a difference in the amount of tissue consumed, but by the consumption of different tissue. Feeding by the moth in the lower taproot did not affect *C. maculosa* biomass likely because new root growth above the feeding site compensated for the putative reduction in water and nutrient uptake caused by herbivory. On the other hand, feeding by the weevil in the vascular tissue reduced shoot biomass likely because compensation above the feeding site was anatomically constrained by the destroyed xylem vessels (Steinger and Müller-Schärer 1992). In the present study, we did not observe an increase in main root biomass in plants exposed to fine root-feeding wireworms, indicating that common milkweed responses to fine root damage differ from the pattern of compensation mentioned above. However, our result that main root-feeding by *T. tetraophthalmus* decreases shoot biomass is consistent with Steinger and Müller-Schärer's inference about destroyed transport cells.

These patterns highlight the relevance of studying belowground herbivory at a finer spatial scale than has typically been investigated. Using split-pot experiments, Robert et al. (2012a) demonstrated both localized and systemic induced responses within root systems of maize (Zea mays, L., Poaceae) plants exposed to belowground herbivory by *Diabrotica virgifera virgifera* (LeConte, Chrysomelidae) larvae. This study, together with the results presented here, suggests a need for increased attention to individual root types and within-root system responses, instead of treating belowground plant material as a single mass of undifferentiated tissue. While our method of assessing root damage—measuring the length of each necrotic section—is more accurate than some traditional methods such as slant boards and visual scales, it, too, has limitations. Because main and fine roots differ in mean diameter, a centimeter of damage to a main

root usually reflects the removal of more tissue than the same length of damage to a fine root. To avoid the limitations of length-based approaches, future work could employ newer techniques, such as acoustic detection, mini-rhizotrons, and x-ray microtomography, which provide non-destructive means of observing belowground insect behavior and quantifying the amount of tissue consumed (Mankin et al. 2008).

Specific impacts of root-feeding insects also may be explained by the physiological consequences (and associated impacts on up- and downstream tissue) of different types of feeding. Boring damage to main roots often removes (A. Erwin, *personal observations*) or compromises the function of the vasculature, putatively reducing transport to shoots, and explaining the decrease in shoot biomass reported here. External damage to fine roots is less likely to produce such a pattern; common milkweed fine roots have smaller vascular bundles than main roots (A. Erwin, *unpublished data*). Thus, we suggest that different physiological impacts of different types of damage may be another factor explaining why main root feeding by *T. tetraophthalmus* decreased shoot biomass whereas fine root feeding by wireworms did not.

A recent meta-analysis demonstrated that the magnitude of the effect of belowground herbivory on root biomass is predicted by insect guild: overall, external chewers cause greater root losses than borers (Zvereva and Kozlov 2011). We detected different impacts of a chewing *versus* a boring insect, but our results are not consistent with this general pattern. Damage by wireworms did not reduce any component of plant biomass whereas damage by *T. tetraophthalmus* reduced main root and fine root biomass. One consideration is that not all wireworms feed exclusively by chewing.

Agriotes spp. bite the root and then suck at the point of damage. Seventeen percent of our wireworm collection was comprised of *Agriotes mancus*. If this mode of feeding is common in *H. abbreviates* (83% of the collection), then this may explain why our findings do not follow the conclusion of the meta-analysis (Zvereva and Kozlov 2011).

Finally, diet breadth may play a role in explaining the species-specific pattern of root damage reported here. Robert et al. (2012b) recently showed that specialist root-feeding larvae (*Diabrotica virgifera virgifera*) preferred and grew best on the nutrient rich, yet well-defended crown roots of maize whereas two generalist herbivores (*Diabrotica balteata* LeConte, Chrysomelidae and *Spodoptera littoralis* Boisduval, Noctuidae) were deterred from feeding on these tissues.

Effects of insect density and soil nutrients. The density of root-feeding insects may influence patterns of belowground herbivory via effects on host location, feeding behavior, growth rates, sex ratios, and survival (Brown and Gange 1990, Whittaker 2003, Onstad et al. 2006, Robert et al. 2012a). We tested how insect density influences root damage under typical (non-outbreak) conditions to determine whether density dependence attenuates the negative effects of herbivores on plants. We found that damage to main roots was positively correlated with overall insect density, and increased with increasing density of *T. tetraophthalmus*. Damage to fine roots was also positively correlated with overall density, and increased with the density of wireworms. Previous experiments (e.g., Spike and Tollefson 1988) have demonstrated an interactive effect of insect density and soil nutrients on root damage, yet our study is one of the first to show that the interaction itself may depend on root type; here, damage increased with insect density under high, but not low, soil nutrients for main roots only.

Increasing insect density decreased all components of plant biomass (shoots, main roots, and fine roots) overall, but reductions were most pronounced in main roots exposed to *T. tetraophthalmus*. Indeed, when we excluded the *T. tetraophthalmus* data and again tested for the effects of density, nutrients, and their interaction on plant biomass, we found that only nutrients remained significant. This result indicates that the effects of density, identity-by-density, and identity-by-nutrients on plant damage and biomass were driven by the overwhelming influence of *T. tetraophthalmus*.

The level of nutrients in the soil may mediate patterns of belowground herbivory by influencing the quantity (e.g., production, lifespan) and quality (e.g., defenses, nitrogen content) of plant roots (Stevens et al. 2007, Johnson et al. 2009). We predicted that high soil nutrients would increase overall plant biomass as well as plants' ability to maintain biomass when damaged, and that these effects would vary by plant part and insect species. All components of plant biomass increased under high nutrients, as expected. Damage by *T. tetraophthalmus,* but not wireworms, constrained the ability of main and fine roots to increase biomass in response to added soil nutrients. Moreover, high nutrients affected the mass of individual wireworms and *T. tetraophthalmus* larvae that were feeding belowground. This result potentially suggests a plant-mediated indirect effect of soil nutrients on insect performance, however it is important to note that because fertilized soil and root systems were contained in the same pots, we cannot distinguish between direct and plant-mediated effects of the low and high fertilizer treatments. Split pot experiments would facilitate the identification of treatment- *versus* plant-mediated effects. This experimental approach is difficult in common milkweed because most genotypes produce a single main root (A. Erwin, *unpublished data*),

which cannot be subdivided.

Belowground herbivory may alter resource allocation between different types of roots, potentially affecting the performance of larvae that feed on these different tissues. Studies using radioisotopes have shown resource allocation away from the site of damage and to distal tissues that are not easily accessible to herbivores (reviewed in Orians et al. 2011). This process, termed induced resource sequestration, may be effective as a 'safeguarding' strategy, but may also be associated with ecological costs (Orians et al. 2011). For example, such allocation may make enriched tissues increasingly vulnerable to attack by other species. Here, we demonstrate that *T. tetraophthalmus* larval mass increased under high nutrients significantly more in the presence of wireworms than in the presence of only conspecifics. If in common milkweed photosynthates are diverted away from the site of damage (e.g., fine roots) to other undamaged (fine and main root) tissues, then fine root attack by wireworms may have lead to increases in main root quality and, in turn, the observed increase in *T. tetraophthalmus* larval mass. That we did not detect an increase in wireworm mass in response to *T. tetraophthalmus* feeding (regardless of nutrient level) may possibly be explained by structural limitations in signal transport, i.e., vascular flow may be limited to one direction.

Insect-insect interactions. Because plant roots are often simultaneously attacked by different insects, belowground intra- and interspecific interactions may be common. In this study, conspecific density was a strong negative predictor of the mass of wireworms, but did not affect the mass of the specialist *T. tetraophthalmus*, as predicted. The survival of both insects was negatively correlated with conspecific

density, in contrast to a recent study on *Diabrotica virgifera* that demonstrated a hump-shaped correlation between larval density and performance (Robert et al. 2012a). The shape of density dependence was species specific, as shown in a comparison of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) and the northern corn rootworm (*Diabrotica barberi* Smith and Lawrence) (Onstad et al. 2006). We also found that the presence of the herbivore *T. tetraophthalmus* decreased, rather than increased, the survival (but not mass) of omnivorous wireworms. The decrease in wireworm survival when the two insects co-occurred potentially could be explained by (i) the significant reduction in the predominant food source (i.e., fine roots) of wireworms in response to main root feeding by *T. tetraophthalmus*, and/or (ii) a stronger induction of chemical defenses (cardenolides) in *A. syriaca* roots by *T. tetraophthalmus* damage than by wireworm damage. Furthermore, *T. tetraophthalmus* survival and mass were not reduced in the presence of wireworms. One explanation for the lack of evidence of intraguild predation is that *T. tetraophthalmus* larvae sequester root cardenolides (Malcolm 1991, Agrawal et al. 2012). If *A. syriaca* roots have lower concentrations of cardenolides than *T. tetraophthalmus* larvae, then roots may provide omnivores with a more palatable food resource than herbivores. An alternative explanation is that *T. tetraophthalmus* feed internally (A. Erwin, *personal observations*, Williams 1941), a habit that may provide physical protection from predators.

We have demonstrated that insect identity, density, and soil nutrients strongly influence patterns of belowground herbivory in common milkweed. Species-specific root damage had differential impacts on below- and aboveground plant biomass. Density dependence was generally important, affecting damage and the mass of both

plants and root-feeding insects, whereas heterospecific interactions between insects appear to have lesser influence. Soil nutrients mediated the impacts of insect identity and density, rather than acting as an independent driving force.

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CHAPTER TWO

ABOVEGROUND HERBIVORY FACILITATES ABOVE- AND BELOWGROUND CONSPECIFIC INSECTS AND REDUCES FRUIT PRODUCTION

Submitted to *Ecology* as Alexis C. Erwin, Tobias Züst, Jared G. Ali, and Anurag A. Agrawal. Aboveground herbivory facilitates above- and belowground conspecific insects and reduces fruit production.

ABSTRACT

Initial herbivory and induced plant responses have the potential to shape patterns of subsequent insect attack above- and belowground. When different life stages of the same herbivore species damage different plant parts sequentially, there is especially strong potential for induced effects because the sequence of attack is predictable for the plant. We studied herbivory by foliage-feeding adults and root-feeding larvae of the red milkweed beetle (*Tetraopes tetraophthalmus*) on common milkweed (*Asclepias syriaca*) to address plant-mediated consequences of above- and belowground herbivory for species interactions. We found that the inducibility of two defensive traits, cardenolides and latex, was localized to the damaged subsystem (root *vs.* shoot), rather than being induced systemically. We next tested for the effects of above- and belowground herbivory on damage by, and the performance of, later-feeding larvae. Attack by adults aboveground increased root damage and larval survival, suggesting an increase in root quality following induction in shoots. Attack by larvae belowground did not affect the performance of later-feeding larvae, indicating limited importance of both induced root cardenolides and competition between clutches for these herbivores. In a field experiment, aboveground herbivory facilitated damage by other *T. tetraophthalmus* adults and milkweed leaf beetles (*Labidomera clivicollis*), and ultimately reduced fruit production by 33 %. However, aboveground herbivory reduced the probability of damage by monarch (*Danaus plexippus*) caterpillars, likely indicating avoidance of *T. tetraophthalmus* adult damaged plants by adult monarchs. We found no evidence for enhanced recruitment of entomopathogenic nematodes in the rhizosphere of above- or belowground damaged plants. We conclude that induced responses to

aboveground damage by adult *T. tetraophthalmus* not only facilitate further damage by adults, but enhance performance of their root-feeding larvae, most likely as a result of host plant manipulation.

KEYWORDS

Asclepias syriaca; cardenolide; common milkweed; entomopathogenic nematodes; induced response; latex; red milkweed beetle; plant resistance; *Tetraopes tetraophthalmus*

INTRODUCTION

It is well understood that insect feeding can systemically alter a plant's physiology. Early work on plant-mediated species interactions focused almost entirely on foliage feeding herbivores (Karban and Baldwin 1997). More recently, it has become apparent that insect herbivores sharing the same host plant but utilizing different subsystems (i.e., shoots *vs.* roots) may also be linked via plant-mediated induced responses (Wardle et al. 2004a, van der Putten et al. 2009, Soler et al. 2012). Most studies investigating above- and belowground induced plant responses have not explicitly distinguished between simultaneous *versus* sequential damage, and largely have been based on different species attacking the two subsystems (e.g., Masters and Brown 1992, Hunt-Joshi et al. 2004, Rasmann and Turlings 2007, Huang et al. 2012). While such interspecific interactions are important and may be common, we currently lack a general predictive model for their outcome (Kaplan et al. 2008, Johnson et al. 2012), possibly due to the high level of stochasticity in the interaction between any two

species that are only connected by a shared host plant.

As a special case of above- and belowground interactions, a variety of herbivores utilize a single host plant but rely on different tissues at different points in their ontogeny, e.g., adults feeding on leaves and larvae feeding on roots (Rasmann and Agrawal 2008). As such, the location and timing of damage in different subsystems are likely highly predictable for the plant (Karban et al. 1997), and a coordinated plant response should be advantageous. We would thus predict that the early-season life stage of an herbivore should induce resistance in the plant part predictably attacked by the later-season life stage, resulting in benefits to the plant. Alternatively, the herbivore might manipulate the plant to increase performance of its kin, in which case damage by the early-season life stage should increase the quantity and/or quality of the plant tissue that will be eaten by the later-season life stage (i.e., induced susceptibility / facilitation).

The red milkweed beetle *Tetraopes tetraophthalmus* Foster (Cerambycidae) is a univoltine specialist herbivore on common milkweed *Asclepias syriaca* L. (Apocynaceae). Early in the season, adults are present aboveground, with females laying multiple clutches in mid-summer. Neonates find their way into the soil and begin to feed on milkweed roots and rhizomes, with larvae and adults co-occurring in the peak of the growing season (Agrawal 2004). In response to above- and belowground attack by the community of milkweed herbivores (≈10 species; Matter 2001, Van Zandt and Agrawal 2004a, Rasmann et al. 2009a), common milkweed induces a well-characterized suite of defenses (Agrawal 2005). Thus, the *Tetraopes*-milkweed system represents an ideal model to examine how plants respond to sequential herbivory and if these responses differ within and among herbivore species.

Using a series of field experiments, we investigated the single and interactive effects of above- and belowground damage by *T. tetraophthalmus*. Specifically, we asked (1) how above- and belowground herbivory affect cardenolide concentrations and latex exudation in shoot and root tissues; (2) if aboveground herbivory by adults impacts belowground herbivory by larvae and *vice versa*, and if above- and belowground herbivory impact the survival of subsequent larvae; and (3) if early-season above- and belowground herbivory influences host plant use by later-season aboveground insects, soil-dwelling entomopathogenic nematodes (EPN), and plant performance.

METHODS

Plants. Common milkweed *A. syriaca* is a clonal perennial plant found in disturbed areas and early successional habitats across eastern North America. In central New York, USA, ramets typically emerge in late May and flower from mid-June through July. Observations have revealed that the system of vascular roots includes a persistent 'main' root (mean diam. 4.5 ± 1.1 mm, usually third order) and most belowground herbivory by red milkweed beetle larvae occurs on this root type, rather than on 'fine' roots (mean diam. 0.5 ± 0.25 mm, first and second order) (Erwin et al. 2013).

Insect herbivores. The red milkweed beetle *T. tetraophthalmus* is a monophagous herbivore of common milkweed. At our field site in central New York, USA, adult beetles emerge from the soil in late June and begin to feed on flowers and foliage. Females oviposit clutches of 10 - 15 eggs in dry stems of nearby grasses and

forbs (Gardiner 1961). Larvae hatch after approximately 6 - 10 days, drop to the ground, and begin to feed on milkweed roots and rhizomes (Agrawal 2004). Adults and larvae typically only co-occur on individual milkweed ramets from mid to late July.

In addition to *T. tetraophthalmus*, nine species of herbivores are commonly observed at our field site: three homopterans (*Aphis asclepiadis*, *A. nerii*, *Myzocalis asclepiadis*), two lepidopterans (*Danaus plexippus*, *Euchaetes egle*), two hemipterans (*Lygaeus kalmii*, *Oncopeltus fasciatus*), a coleopteran (*Labidomera clivicollis*), and a dipteran (*Liriomyza asclepiadis*) (Van Zandt and Agrawal 2004a, A. Erwin, *personal observations*).

Experimental materials. Plants used to test for induced cardenolides (Experiment 1) and induced latex, plant damage, and insect performance (Experiment 2) were grown in May 2009 using seeds collected from ten patches (not known to be distinct clones) occurring in an old field in Tompkins Co., New York, USA. Seeds were cold stratified at 4°C on moist filter paper for a week, nicked, and germinated in the dark at 26° C. Seedlings were then planted in commercial potting soil (Metro-Mix, Sun Gro Horticulture, Canada CM Ltd.) in plastic pots (10 cm diameter) and grown in a growth chamber (12:12 D/N light, 26:20 °C D/N temperature). After two months, plants were moved to larger (3.8 L) plastic pots and placed outside for two years. In May 2011, individual plants of each family that were $\geq$ 10 cm tall were randomly assigned to Experiment 1 or 2 and then individually enclosed in spun polyester mesh bags to enclose any treatment herbivores and prevent natural colonization by other insects. Plants were watered *ad libitum* and received no fertilizer.

Plants used to test for the impacts of above- and belowground herbivory on the

herbivore community, entomopathogenic nematodes, and plant fitness (Experiment 3) were selected in June 2011 from 20 naturally occurring patches in the same field where seeds had been collected for Experiments 1 and 2. Patches were differentiated based on proximity and density of the ramets as well as several morphological traits (A. Erwin, *unpublished data*). Selected ramets were undamaged, ≥ 5 m apart, 100 - 120 cm tall, and had between 3 - 5 umbels.

To impose herbivory treatments, we used wild-caught adults and laboratory-reared larvae of *T. tetraophthalmus*. We collected adults from patches in the same field and kept them in ventilated containers (30 cm × 20 cm × 15 cm) under natural light at ambient humidity. We provided them with common milkweed leaves for food and dry grass stems as oviposition substrate. We removed the substrate every two days and incubated it in the dark at 30°C for 7 - 10 days (Rasmann et al. 2009a). First-instar larvae were kept without food on moist filter paper in petri dishes (10 cm diameter) for < 24 hrs before being transferred to experimental plants.

Experiment 1: Local and systemic cardenolide induction. To test for the single and interactive effects of above- and belowground herbivory on the induction of cardenolides in milkweed shoots and roots, a 2 × 2 factorial experiment was established in June 2011 using 2-year old potted plants (n = 56; 14 plants/treatment). Treatments consisted of an undamaged control (Con), one *T. tetraophthalmus* adult added to impose aboveground herbivory (Abv), five *T. tetraophthalmus* larvae added to impose belowground herbivory (Blw), and one adult and five larvae added to impose above- and belowground herbivory (Abv+Blw). Plants were individually enclosed in mesh bags, which allowed adults to move and feed freely but not to leave the plant.

An adult feeding event usually results in the removal of the tip of one young leaf. Because the amount of tissue removed is fairly consistent (1.1 ± 0.16 cm^2 per feeding event, n = 15), we quantified aboveground herbivory as the number of leaves that received tip damage. We checked plants daily and removed the adult beetles once ~10 % of the leaves had been damaged (on average 6 ± 0.5 days). Belowground herbivory was imposed to each plant in the Blw and Abv+Blw treatments by digging five 1 cm deep holes evenly spaced on a circle (5 cm diam.) around the plant stem and placing a single first-instar *T. tetraophthalmus* larva in each hole. Larvae were applied two days before adults and harvested ten days later. Based on the results of an earlier experiment (Erwin et al. 2013), we expected that this combination of larvae/days would result in ~10 % of the length of the main root being damaged. These levels of above- and belowground herbivory are within the range commonly observed in the field.

We harvested all of the plants ten days after applying the larvae (= eight days after applying adults). Latex exudation in leaves was measured by cutting the tip (last 1 cm) of the youngest, fully expanded, undamaged leaf and soaking up exuding latex onto a pre-weighed disc (1 cm diam.) of filter paper, which was frozen for later weighing. This measure of exudation likely reflects what feeding insects experience on plants in the field, and has been shown to correlate negatively with the growth of individual milkweed herbivores (Van Zandt and Agrawal, 2004a).

Belowground root material was washed in water and separated from soil. All plant material was oven dried at 40˚C for three days, separated into shoots, main roots, and fine roots, and then ground to powder on a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA). The total cardenolide concentration of shoots and main roots

was measured by analyzing 100 mg of each powder via HPLC (Rasmann et al. 2011). Fine roots were not analyzed because *T. tetraophthalmus* do not cause substantial damage to this tissue (Erwin et al. 2013). We recognize that herbivore damage to the main root may result in induced defenses in attached fine roots. However, our recent work on the spatiotemporal pattern of cardenolide induction within the root system of individual plants (A. Erwin, *unpublished data*) is beyond the scope of this study. To assess variation in latex exudation, and shoot and root cardenolides, we employed two-way ANOVAs with aboveground herbivory and belowground herbivory as fixed main effects.

Experiment 2: Individual plant and insect performance. We conducted a bioassay experiment to test for the effects of initial above- and belowground herbivory on latex exudation, plant damage, and the survival and mass of later-feeding larvae. Again, we applied 2 × 2 factorial treatments to 2-year old potted plants (n = 80; 20 plants/treatment) that were individually bagged in mesh. Treatments were the same as in Experiment 1 except that we only used two larvae per plant to impose the belowground herbivory treatment in order to limit impact on subsequent bioassay larvae. Eight days after applying the treatment larvae (= six days after applying adults), we removed the bags and adults, and then applied ten freshly hatched bioassay larvae to each plant. Bioassay larvae were individually added as in Experiment 1. Plants were allowed to grow for ten more days and were protected from additional herbivory by a large mesh cage. We then harvested plants and measured several aboveground traits (total ramet height, total number of leaves, and number of damaged leaves) and belowground traits (total length of the main root, damaged length of the main root, and

main root mass) as well as individual larval survival and mass. All plant traits and insect survival were analyzed using two-way ANOVAs to test for the effects of above- and belowground herbivory.

Total and damaged number of leaves were counted to estimate aboveground herbivory. We then separated above- and belowground tissues, washed root systems, and separated main and fine roots. The total main root length and length of damage were measured to estimate belowground herbivory (as in Erwin et al. 2013). We dried main roots at 40˚C for three days and weighed them to the nearest mg. Surviving *T. tetraophthalmus* larvae were counted, weighed, and allocated to initial herbivory treatment or bioassay (Fig. S2.3). Unrecovered larvae were presumed dead.

To investigate whether the addition of belowground larvae altered aboveground damage by adults, we excluded plants belonging to the Con and Blw treatments and analyzed the effect of belowground herbivory on the number of leaves damaged in Abv and Abv+Blw plants using a one-way ANOVA, including total leaf number as a covariate. Correspondingly, to evaluate whether the addition of aboveground adults altered belowground damage by larvae, we excluded plants in the Con and Abv treatments and tested for an effect of aboveground herbivory on damage to the main roots of Blw and Abv+Blw plants, including the total length of the main root as a covariate.

Experiment 3: Later-season above- and belowground impacts. To test for the effects of initial above- and belowground herbivory on leaf damage by, and the abundance of, subsequent aboveground insect herbivores as well as EPN and plant performance, we applied Abv and Blw treatments in a factorial design to naturally

occurring field plants (n = 80; 20 plants/treatment, see *Experimental materials*). We randomly assigned treatments to four similar plants per patch in a total of 20 patches and enclosed the shoots of all plants individually in mesh bags. Treatments were the same as in Experiment 1 except that we increased the number of adults (from 1 to between 2 – 4) and the number of larvae (from 5 to 20) because plants were significantly larger (~100 cm tall at the start of the experiment) than the potted plants used in Experiments 1 and 2; 2 – 4 adults were used to impose the target damage level (~10 %), which was achieved by 2.5 ± 0.5 days on average. *T. tetraophthalmus* dispersal is quite limited and previous work has demonstrated that individual adults may remain in a patch for several days under natural conditions (McCauley et al. 1981). To simulate the natural phenology of the herbivores, larvae were applied in two groups to each plant (10 larvae per "clutch"), separated by 10 days (Fig. S2.1). First observations were made 10 days after the second group of larvae was added and mesh bags had been removed (Fig. S2.1). We recorded all leaf damage caused by subsequent naturally colonizing herbivores.

Starting on 22 July 2011, we surveyed plants weekly for leaf damage and counted leaf number and the abundance of all insect herbivores present on each plant. We were able to differentiate among leaf damage caused by *T. tetraophthalmus*, *L. clivicollis*, *E. egle*, *D. plexippus*, and *L. asclepiadis* since these herbivores cause easily recognizable species-specific leaf damage. However, damage by *E. egle* and *L. asclepiadis* was too sparse for a meaningful analysis and is therefore not reported. Surveys were ended after eight weeks, when plants began to senesce and insect populations declined. In early October, when fruits had fully matured, we measured the

number of all fruits on each plant as an estimate of sexual reproduction.

To calculate the mean leaf area removed for different damage types, we collected and scanned leaves from plants growing in the same field that had naturally received only one type of damage (n = 20 leaves/damage type/species). Scanned leaves were used to generate mean areas per damage type (*T. tetraophthalmus* tip: 1.08 cm^2; *T. tetraophthalmus* side: 8.99 cm^2; *D. plexippus* center: 0.78 cm^2; *D. plexippus* edge: 12.32 cm^2) and weekly damage counts from the field were multiplied by these means.

All analyses of the field experiment were fit using the statistical program R (R Development Core Team, 2012) and the packages *nlme* 3.1-104 and *lme4* 0.999999-0. Damage by the different herbivore species was analyzed using a set of mixed-effects models with identical structure. Plant identity was treated as random effect to account for repeated measures over eight weeks, and was nested within patch. In each full model, aboveground herbivory, belowground herbivory, week, and all interactions were fitted as fixed effects and leaf number was fitted as a covariate. The *lme4* package used for non-normal data does not provide F-tests (Gelman and Hill 2007). We therefore followed a model simplification approach to identify the single most parsimonious model for each herbivore by comparing nested models using Akaike's information criterion (AIC). For each herbivore species, we selected the model with the lowest AIC, favoring simpler models when pairs of nested models were tied with AICs within two units per difference in the number of parameters. To test for significance of treatment differences, we constructed 95 % confidence intervals (CIs) for parameter estimates as recommended by Gelman and Hill (2007). We present these parameter estimates on the scale of the linear predictor with their 95 % CIs and an approximate *p*-value, based on

resampling of the posterior distribution (Gelman and Hill 2007).

Within the mixed-model framework, we selected the models most appropriate for the types of data. Damage caused by *T. tetraophthalmus* and *D. plexippus* was analyzed in two steps: first we analyzed occurrence (presence or absence = probability) of damage using models with a binomial error structure (function *glmer* in *lme4*), and second we removed all zeros and analyzed the log-transformed data using Gaussian models (function *lme* in *nlme*). The number of damage marks caused by *L. clivicollis*, was analyzed using a model with a poisson error structure (function *glmer* in *lme4*) which is appropriate for count data.

To investigate whether above- and belowground herbivory and plant patch affected the abundance of EPN, an agent of indirect defenses, we buried cages containing EPN sentinel larvae in the field as described in Ali et al. (2012). Each cylindrical cage (7 cm length × 3 cm diam.) was filled with autoclaved sand (10 % moisture) and one late instar larva of the greater wax moth *Galleria mellonella* L. (Pyralidae) (GrubCo©, Fairfield, OH, USA). One cage was buried 15 cm below the base of each experimental plant in 12 of the 20 patches used in Experiment 3, resulting in 12 replicate cages per treatment. After 4 days, we recovered cages, rinsed larvae, and placed them on moistened filter paper in individual Petri dishes. We confirmed EPN infection (by infective juvenile emergence from cadavers) and recorded EPN-inflicted larval mortality 0 to 48 h after removal from soil. We used chi-square tests of independence to test the effects of above- and belowground herbivory and plant patch (block) on *G. mellonella* mortality.

RESULTS

Experiment 1: Local and systemic induced responses. Induction following aboveground herbivory was restricted to aboveground tissues: shoot cardenolides increased by 20.6 % (Abv: $F_{1, 50} = 5.10$, $p = 0.028$; Fig. 2.1A; Blw: $F_{1, 50} = 0.55$, $p = 0.461$) and there was no interaction (Abv × Blw: $F_{1,50} = 0.94$, $p = 0.336$). Belowground herbivory significantly increased root cardenolides by 21.2 % (Blw: $F_{1, 52} = 4.55$, $p = 0.038$; Fig. 2.1B) and led to a marginally significant increase in shoot cardenolides of 17.4 % (Abv: $F_{1, 52} = 3.19$, $p = 0.080$). Again, there was no interaction (Abv × Blw: $F_{1, 52} = 0.93$, $p = 0.338$). As with cardenolides, we found evidence of localized induction in latex, which increased by 43.7 % in plants that were damaged aboveground compared to plants that were not (Abv: $F_{1, 75} = 7.45$, $p = 0.008$; Fig. 2.1C). We did not detect a change in latex exudation due to belowground herbivory (Blw: $F_{1, 75} = 0.00$, $p = 0.998$) and there was no interaction (Abv × Blw: $F_{1, 75} = 2.05$, $p = 0.156$).

Experiment 2: Individual plant and insect performance. Herbivory treatments did not significantly impact total ramet height (full model, $F_{1, 75} = 0.94$, $p = 0.428$), but did reduce belowground root biomass (full model, $F_{1, 73} = 3.62$, $p = 0.017$). Belowground herbivory reduced main root mass by 31 % compared to the control (Blw: $F_{1, 73} = 4.51$, $p = 0.037$). Main root mass also tended to be lower with aboveground herbivory and the dual herbivory treatment (28 % and 30 % reductions, respectively, compared to the control), although these effects were marginally significant (Abv: $F_{1, 73} = 2.94$, $p = 0.091$; Abv × Blw: $F_{1, 73} = 3.43$, $p = 0.068$).

In the subset of plants receiving aboveground damage, we found a marginally significant facilitative effect of belowground herbivory on damage by adults

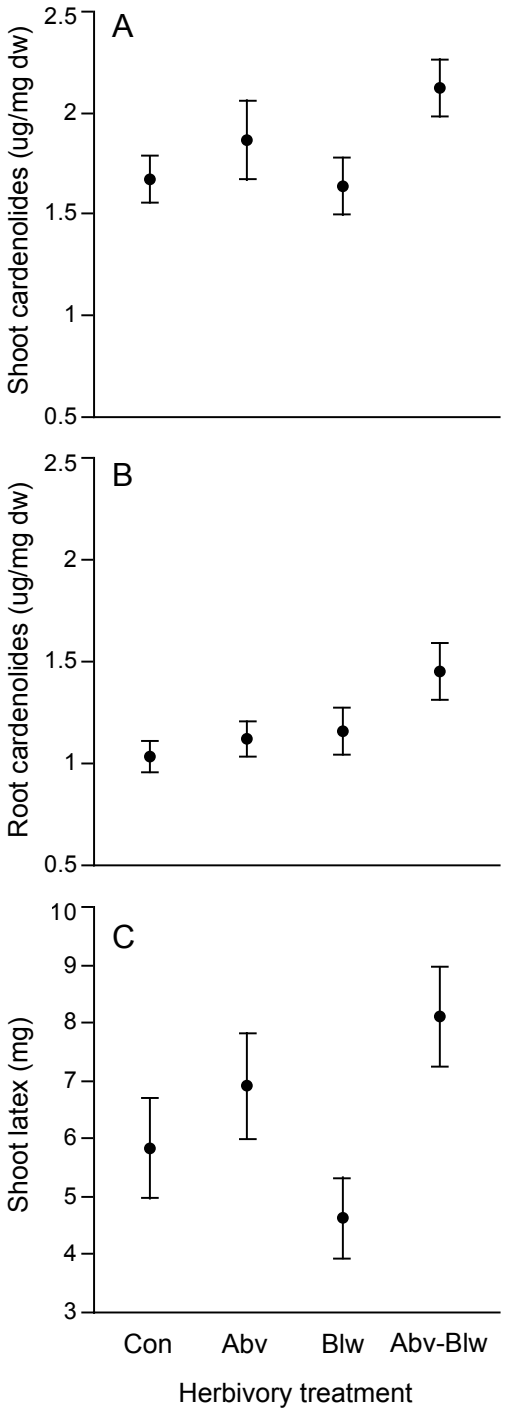

Figure 2.1 Impact of initial aboveground herbivory by adult, and belowground herbivory by larval, *Tetraopes tetraophthalmus* on the concentration of cardenolides in the (A) shoots and (B) main roots, and the (C) exudation of latex in the shoots of *Asclepias syriaca* plants. Treatments consist of Con: undamaged control; Abv: aboveground herbivory only; Blw: belowground herbivory only; Abv+Blw: above and belowground herbivory. Values are ± 1 SE.

($F_{1, 36} = 3.03$, $p = 0.090$; Fig. 2.2A). Plants with more leaves suffered more damage (Tot. lvs: $F_{1, 36} = 6.66$, $p = 0.014$; Fig. 2.2A), but this effect was independent of the herbivory treatment (Tot. lvs × Blw: $F_{1, 36} = 0.48$, $p = 0.492$). Aboveground herbivory by adults facilitated belowground damage by larvae ($F_{1, 36} = 5.64$, $p = 0.023$; Fig. 2.2B), and plants with a higher total root length suffered more damage (Tot. length: $F_{1, 36} = 7.34$, $p = 0.010$; Fig. 2.2B), but again this effect was independent of the herbivory treatment (Tot. length × Abv: $F_{1, 36} = 2.27$, $p = 0.141$).

We recovered 26.3 % of the larvae added to impose the belowground herbivory treatment and 35.0 % of the subsequently added bioassay larvae (Figure S2.3). These recovery rates are similar or higher than those reported in other studies in this system (Rasmann et al. 2011, Rasmann and Agrawal 2011, Erwin et al. 2013). Aboveground herbivory did not impact the survival of treatment larvae (Abv: $F_{1, 17} = 0.05$, $p = 0.822$), yet the survival of bioassay larvae was 64.2 % higher on plants that had been previously damaged aboveground compared to those that had not, regardless of previous belowground damage (Abv: $F_{1, 68} = 11.20$, $p = 0.001$; Blw: $F_{1, 68} = 0.41$, $p = 0.523$; Abv × Blw: $F_{1, 68} = 0.53$, $p = 0.468$; Fig. 2.3). A model including above- and belowground herbivory and the interaction did not predict the mass of surviving bioassay larvae (full model: $F_{1, 65} = 1.76$, $p = 0.164$), and the addition of larval survival did not improve the model fit ($F_{1, 61} = 1.77$, $p = 0.111$).

Experiment 3: Later-season aboveground impacts. Initial aboveground herbivory by *T. tetraophthalmus* adults affected subsequent damage by several species (Table S2.1). First, damaged plants were 19.8 % more likely to be damaged further by naturally colonizing *T. tetraophthalmus* adults (parameter estimate for the treatment

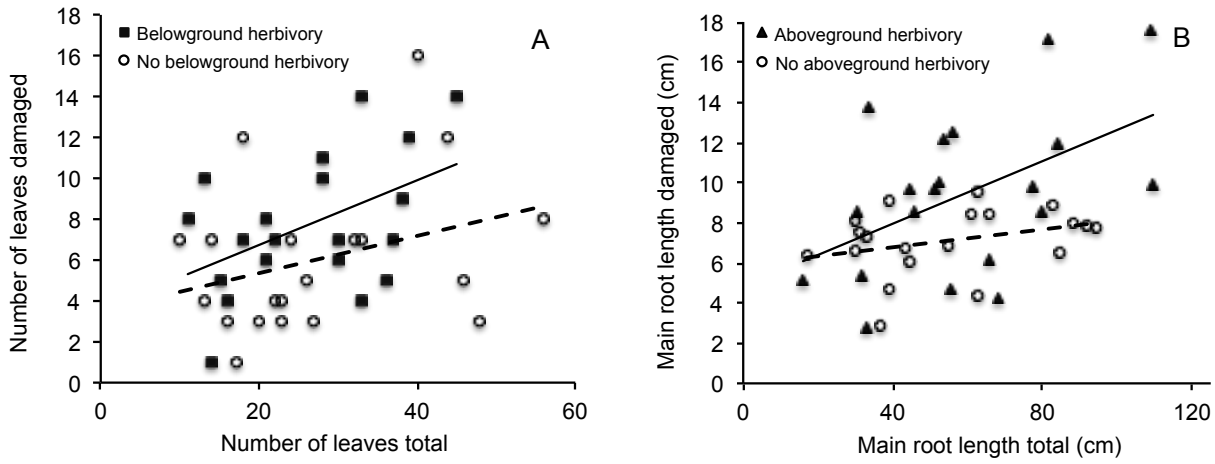

Figure 2.2 (A) Impact of the total number of *Asclepias syriaca* leaves and belowground herbivory by *Tetraopes tetraophthalmus* larvae on the number of leaves damaged by *T. tetraophthalmus* adults. (B) Impact of *A. syriaca* main root total length and aboveground herbivory by *T. tetraophthalmus* adults on the length of damage to main roots by *T. tetraophthalmus* larvae.

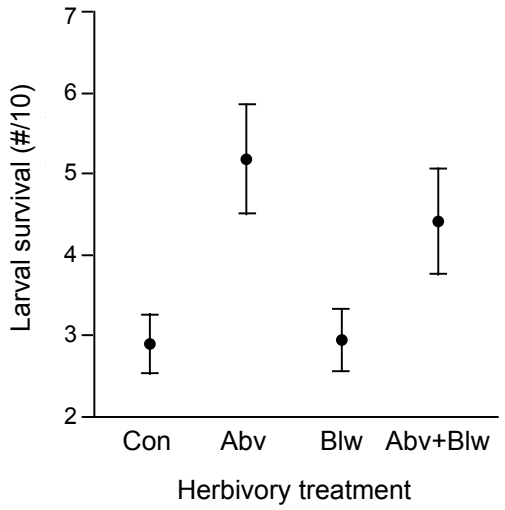

Figure 2.3 Impact of initial aboveground herbivory by adult, and belowground herbivory by larval, *Tetraopes tetraophthalmus* on the survival of *T. tetraophthalmus* bioassay larvae that were added to roots of the same *Asclepias syriaca* plants. Treatments consist of Con: undamaged control; Abv: aboveground herbivory only; Blw: belowground herbivory only; Abv+Blw: above- and belowground herbivory. Values are LS means ± 1 SE.

effect relative to control, β_{Abv} = +8.01, CI = 2.77 – 13.15, p = 0.002; Fig. 2.4A). The proportion of overall damaged plants decreased throughout the experiment (β_{Week} = -0.34, CI = -0.59 – -0.08, p = 0.006) and treatment differences decreased over time ($\beta_{Abv \times Week}$ = -0.76, CI = -1.49 – -0.06, p = 0.042). In the subset of plants that had some later-season leaf damage (excluding aboveground treatment damage), initial aboveground herbivory was associated with a 125 % increase in damage by *T. tetraophthalmus* adults (Abv: $F_{1, 69}$ = 106.68, p < 0.0001; Fig. 2.4C). Plants exposed to initial aboveground herbivory by *T. tetraophthalmus* also had 80.4 % more leaf damage by *L. clivicollis* than plants that were previously undamaged aboveground (β_{Abv} = +0.59, CI = 0.23 – 0.91, p = 0.004; Table S2.1).

In contrast, plants that were initially damaged aboveground by *T. tetraophthalmus* adults were 41.6 % less likely to receive subsequent leaf damage by *D. plexippus* caterpillars during the first half (four weeks) of the experiment (β_{Abv} = -1.13, CI = -1.93 – -0.32, p = 0.006; Fig. 2.4B). In the second half of the experiment, 100 % of plants were damaged by *D. plexippus*, and the overall amount of leaf damage increased (Fig. 2.4D). However, there was no effect of treatment on the amount of leaf damage (Abv: $F_{1, 47}$ = 1.06, p = 0.308; Fig. 2.4D). Overall, there was no indication for an effect of initial belowground herbivory by *T. tetraophthalmus* larvae on later-season leaf damage, and no support for treatment interactions (Table S2.1).

We observed *T. tetraophthalmus*, *E. egle*, *D. plexippus*, *L. asclepiadis, L. kalmii, O. fasciatus, A. nerii, A. asclepiadis, M. asclepiadis* (but never *L. clivicollis*) during the growing season. Of the three herbivores that were present in sufficient numbers to be analyzed, none deviated from a random distribution among treatments (*L. kalmii*: χ^2 =

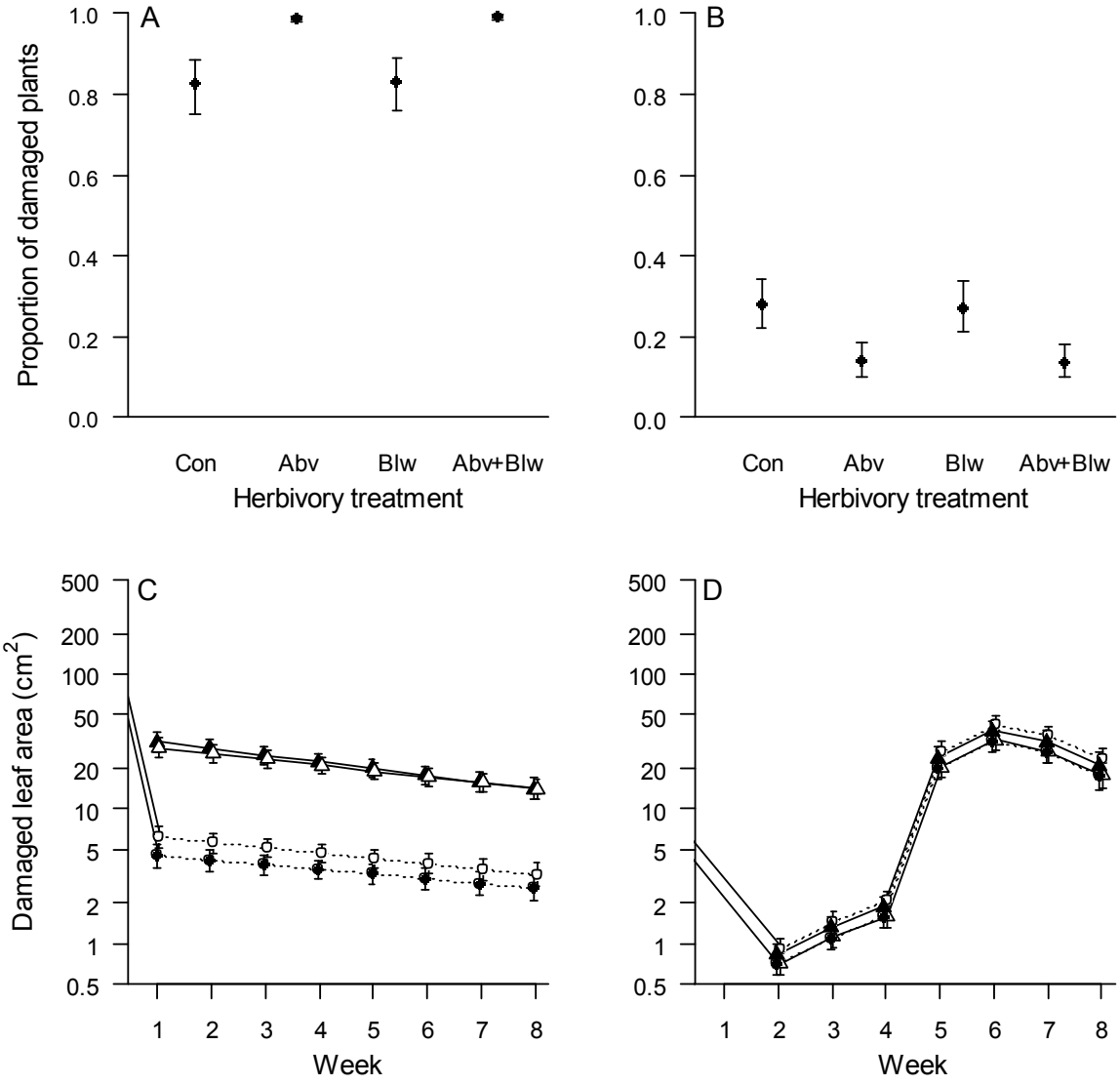

Figure 2.4 Top: Impact of initial aboveground herbivory by adult, and belowground herbivory by larval, *Tetraopes tetraophthalmus* on the probability of subsequent leaf damage by *T. tetraophthalmus* (A) and *D. plexippus* (B). Treatments consist of Con: undamaged control; Abv: aboveground herbivory only; Blw: belowground herbivory only; Abv+Blw: above- and belowground herbivory. Values are whole-season means ± 1 SE. Bottom: Amount of damage caused by *T. tetraophthalmus* (C) and *D. plexippus* (D) throughout the field season. Values are weekly means for Con (white circle), Abv (white triangle), Blw (black circle) and Abv+Blw (black triangle) ± 1 SE. For *D. plexippus*, the first week was excluded due to a lack of data.

2.28, df = 3, $p = 0.513$; *T. tetraophthalmus*: $\chi^2 = 4.41$, df = 3, $p = 0.220$; pooled aphid numbers: $\chi^2 = 3.95$, df = 3, $p = 0.267$).

We next tested for differences in the mortality of *G. mellonella*, a sentinel for EPN in the soil. We recovered 100 % of the 48 *G. mellonella* sentinels we added (1 per plant) and 42 % were infected by EPN. Infection was caused exclusively by *Steinernema carpocapsae*, a species native to the USA. Infection resulted in variable mortality among treatments (Con: 33 %; Abv: 58 %; Blw: 33 %; Abv+Blw: 42 %), but these rates were not significantly different ($\chi^2 = 2.057$, df = 3, $p = 0.56$). Nonetheless, sentinel mortality marginally differed among plant patches ($\chi^2 = 18.706$, df = 11, $p = 0.067$), indicating spatial variation in EPN within the field.

The net effects of above- and belowground herbivory were negligible on leaf number (Abv: $F_{1, 57} = 0.05$, $p = 0.812$; Blw: $F_{1, 57} = 1.01$, $p = 0.319$; Abv × Blw: $F_{1, 57} = 1.03$, $p = 0.315$). Nonetheless, fruit number was reduced 32.5 % by aboveground herbivory (Abv: Deviance = 5.48, df = 76, $p = 0.019$; Fig. S2.3) but unaffected by belowground herbivory (Blw: Deviance = 1.36, df = 76, $p = 0.243$; Abv × Blw: Deviance = 0.56, df = 76, $p = 0.456$).

DISCUSSION

We demonstrated that above- and belowground herbivory by different life stages of the same insect can have distinct consequences for induced plant responses. Specifically, damage by early-season aboveground adult *T. tetraophthalmus* facilitated the performance of the later-season belowground larvae of the same species, altered host plant use by other species, and the net effect of these interactions decreased *A. syriaca's* reproductive output in the field. Early-season belowground herbivory did not

influence damage by, or the survival of, later-feeding larvae despite induction of cardenolides in roots. These results suggest that the observed interactions between *A. syriaca* and *T. tetraophthalmus* might not be primarily mediated by induction of these well-studied defensive traits, but rather that *T. tetraophthalmus* is able to manipulate its host plant to maximize its own fitness.

Patterns and consequences of induced plant responses. The location and timing of damage by *T. tetraophthalmus* adults and larvae are strongly correlated: most early-season damage occurs aboveground by adults and most later-season damage occurs belowground by larvae. In our study, plants responded to initial aboveground herbivory by inducing defensive traits locally in the shoots at a similar magnitude as previously demonstrated for this system (Rasmann et al. 2009a). However, we found no evidence of systemic defense induction in root tissue. This pattern was also reported by Ali et al. (2011), who showed that aboveground beetle damage by *Diaprepes abbreviatus* (L.) adults failed to induce indirect belowground defense cues that *were* induced by root-feeding larvae of the same species. Because *T. tetraophthalmus* females lay multiple clutches during their lifetimes, it is likely that milkweed root systems are attacked by larvae of different ages/sizes, with individuals present early in the season inducing roots and potentially competing with later larvae. Consistent with our expectation, we found evidence of significant cardenolide induction within root systems as well as a marginally significant increase in shoot cardenolides. These results are concordant with the pattern reported in *Brassica nigra*, where belowground feeding by *Delia radicum* larvae induced glucosinolates locally in roots as well as systemically in shoots (van Dam and Raaijmakers 2006). Based on a meta-analysis of the induced

defense literature, Kaplan et al. (2008) concluded that foliar herbivory generates strong aboveground induced responses, but much weaker belowground responses; conversely, root herbivory tends to elicit responses in leaves and roots of roughly equal magnitude. Despite the fact that these conclusions were largely based on studies of above- and belowground herbivory by different insect species, they are consistent with our findings.

Above-belowground interactions between life stages of a single insect species. Above-ground herbivory is known to affect soil biota via plant changes (Bardgett and Wardle 2010) and can vary among both plant and herbivore species (Wardle et al. 2004b). Although a classic conceptual model (Masters et al. 1993) proposed negative effects of aboveground herbivores on the performance of belowground herbivores, evidence for this pattern has been mixed (Johnson et al. 2012). The findings of our bioassay and field experiments show that initial aboveground herbivory by *T. tetraophthalmus* adults had positive effects on belowground larvae, indicating induced susceptibility (i.e., increased root damage and individual survival). Some plants respond to herbivory by altering growth patterns to compensate for damage. In the present experiment, we speculate that facilitation of *T. tetraophthalmus* larvae by initial aboveground herbivory likely was achieved via increased root quality (nutrition) rather than quantity (biomass) because we observed a marginally significant decrease in main root biomass following aboveground herbivory. Schwachtje et al. (2006) showed that aboveground herbivory increased allocation of primary metabolites to roots. Moreover, the term induced resource sequestration has been proposed to denote the process of herbivores inducing plant signals that result in increased concentrations of primary

metabolites in storage tissues (Orians et al. 2011). Although enhanced storage belowground may be beneficial to plants under some conditions, in our case it may be facilitating root-feeding larvae. Additionally, such a shunting of resources may have contributed to the dramatic reduction in fruit production of plants receiving aboveground damage.

Masters et al. (1993) initially predicted positive effects of belowground herbivores on the performance of aboveground herbivores, but most subsequent tests of their predictions found evidence for the opposite relationship, especially for chewing herbivores (Kaplan et al. 2008, Johnson et al. 2012). In contrast, the results of our bioassay experiment would support the prediction of Masters et al. (1993): leaf damage by *T. tetraophthalmus* adults was higher on plants exposed to initial belowground herbivory by larvae, in spite of marginally higher induced shoot cardenolides. Other studies have demonstrated the same pattern for inter-specific species pairs (Gange and Brown 1989, Masters et al. 2001, Poveda et al. 2004), although it is certainly not universal (Moran and Whitham 1990, Salt et al. 1996, Bezemer et al. 2003). Surprisingly, these same patterns did not hold in our field experiment: subsequent leaf damage by *T. tetraophthalmus* adults was found to be independent of initial belowground herbivory. Weak or undetectable effects of initial belowground herbivory on subsequent leaf damage in the field may reflect a greater ability of established field plants to tolerate attack. We note, however, that we did not measure larval survival or root damage in the field, and the intensity of our belowground herbivory treatment may have been less than intended due to predation or poor establishment.

Effects on plant performance. We found that typical levels of aboveground

herbivory (≤ 10 %) decreased fruit production, a component of plant fitness. We acknowledge that common milkweed's fitness is determined by investment in both vegetative propagation and sexual reproduction, typically over many years. Thus, the potential impact of reduced fruit production for the long-term persistence of genets and populations may be modest. Nonetheless, the reduction in fruit production may shape future interactions between shoots and *T. tetraophthalmus* adults if it translates to altered future investment in sexual reproduction and, specifically, the availability of flowers, which are important attractants for adults (Agrawal 2004, Reagel et al. 2002). Correspondingly, the reduction in fruit production could potentially influence future root-larvae interactions if plants reallocate resources away from sexual reproduction aboveground and toward belowground storage and/or vegetative propagation.

Species-specific impacts on other community members. Our experiment on naturally occurring field plants showed that the community-wide effects of early-season herbivory by *T. tetraophthalmus* foliar-feeding adults were stronger than those of root-feeding larvae, and affected subsequent leaf damage. Specifically, we showed that the effects of early-season aboveground herbivory facilitated subsequent damage by *T. tetraophthalmus*. Although we did not determine the sex of adult beetles, sex-specific behavior of adults provides a clear hypothesis for why aboveground damaged plants received more damage: males prefer to feed on leaves with latex deactivation by females, which provides latex-free meals without requisite trenching (Gontijo 2013). Aboveground herbivory facilitated subsequent damage by another beetle, *L. clivicollis*, that might benefit from the same host plant changes (i.e., latex deactivation) caused by *T. tetraophthalmus*. In a previous experiment, monarch damage also induced

susceptibility (increased attack) to *T. tetraophthalmus* adults (Van Zandt and Agrawal 2004a). Thus, deactivated laticfers and associated herbivory may be a general cue for *T. tetraophthalmus* in mate finding and feeding.

In contrast, we found that aboveground herbivory by *T. tetraophthalmus* adults decreased the probability of subsequent damage by *D. plexippus* larvae. Thus, the plant-mediated interactions between monarchs and *T. tetraophthalmus* are highly asymmetrical. Within the subset of plants that received some *D. plexippus* damage, the total amount of damaged area was not affected by *T. tetraophthalmus* herbivory. This result appears to be a consequence of active avoidance of host plants with previous leaf damage by ovipositing adult *D. plexippus* butterflies. The strength of this discriminant behavior is most likely mediated by the abundance of undamaged host plants: in the second half of the field experiment, the discriminant behavior completely disappeared as monarch populations dramatically increased. Indeed *D. plexippus* appears to be quite sensitive to prior damage, as damage by various chewing herbivores has been repeatedly shown to substantially decrease the growth of larvae feeding later on the same host plants (Agrawal 2005, Van Zandt and Agrawal 2004a, 2004b, Rasmann et al. 2009b; Agrawal et al. 2012). Initial above- and belowground herbivory did not impact subsequent damage by several other species we surveyed (i.e., three aphids, leaf miners, two species of seed predators), although these were generally too rare for our results to be conclusive.

The only organisms we surveyed in soil were EPN. Although we did not quantify the number of EPN attracted to specific treatments, we show that the mortality of EPN sentinel larvae was independent of treatment. Thus we can be confident that the

lack of an effect of early-season belowground herbivory on later-season leaf damage, insect abundance, or plant performance is a true negative result, rather than being an artifact of high EPN-inflicted mortality of *T. tetraophthalmus* larvae. We detected a marginal difference in sentinel mortality among patches, which is an indication of the patchy distribution typical of EPN populations (Stuart and Gaugler 1994).

Conclusions and speculation. Although induced defenses are widely acknowledged to be effective and adaptive plant responses to herbivory, we found that induced responses of *A. syriaca* to early-season aboveground herbivory by *T. tetraophthalmus* adults caused increased preference and performance of their own species, increased damage by another beetle species, and reduced fruit production, with only a modest repellant effect against a third herbivore. Looking ahead, we speculate that distinct patterns (facilitation *vs.* resistance) of above- belowground induced responses may emerge from intra-specific comparisons (where different life stages of the same herbivore attack different plant subsystems) and interspecific comparisons. Additionally, we encourage tests of how the natural timing of damage, i.e., precedent aboveground, precedent belowground, or simultaneous attack affects the strength and direction of above-belowground interactions (Erb et al. 2011, Johnson et al. 2012). Specifically, it would be interesting to test whether a short lapse between damage types (as is the natural pattern for many univoltine insect species) has different impacts on plant and insect performance than a long lag (as is common among species with long larval stages). Such an approach would contribute to a broader understanding of when and how plant-mediated interactions between insects shape community dynamics.

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CHAPTER THREE

ABOVE- AND BELOWGROUND PLANT TRAITS, BUT NOT SYNDROMES, PREDICT INSECT HERBIVORE PREFERENCE AND PERFORMANCE

In preparation for *Ecology* as Alexis C. Erwin and Anurag A. Agrawal. Above- and belowground plant traits, but not syndromes, predict insect herbivore preference and performance.

ABSTRACT

Interactions between above- and belowground subsystems regulate the structure and functioning of terrestrial communities. Because plants are a key link between the two subsystems and many ecological interactions are mediated by plant phenotype, characterizing shoot and root traits is critical for understanding community-level interactions. We studied 20 full-sibling genetic families (genotypes) of common milkweed (*Asclepias syriaca*) to evaluate correlations between 14 traits (relating to above- and belowground growth, defense, and ecophysiology) and to address whether genotypes can be clustered according to their trait combinations, i.e., into syndromes. We then asked how traits and syndromes influenced the performance of foliage-feeding adults and root-feeding larvae of the (co)evolved red milkweed beetle (*Tetraopes tetraophthalmus*). Plant traits were generally positively correlated within and between subsystems, and we detected only one trade-off: the length and cardenolide concentration of main roots were negatively correlated. Genotypes formed three distinct clusters, characterized by having a small, cardenolide-rich main root, being large and poor quality (high C:N ratio), or having slightly higher foliar cardenolides and intermediate values of the aforementioned traits. Aboveground herbivory by adult beetles did not vary among clusters, but was marginally affected by four *belowground* traits: root bud number, main and fine root biomass, and main root C:N. Although herbivory by, and the mass and survival of, belowground larvae were also not influenced by cluster, larval emergence from oviposition substrate was significantly higher when females had fed on plants with larger main roots. This surprising result suggests host plant preference by adults is likely mediated by a (currently unknown)

mechanism for detecting the plant's belowground phenotype. Finally, larval performance was independent of larval emergence, indicating that putative preferences of adult females did not result in increased performance of their offspring. We conclude that the significant variation we observed for plant traits organizes genotypes into potential syndromes, but that these potential syndromes do not appear to strongly influence the performance of adult or larval beetles.

KEYWORDS

Asclepias syriaca; cardenolide; cluster analysis; herbivory; induced response; latex; milkweed; plant-insect interactions; plant resistance; red milkweed beetle; *Tetraopes tetraophthalmus*

INTRODUCTION

In terrestrial communities, linkages between aboveground and belowground subsystems can strongly influence the performance of plants and their consumers. Plant shoot and root traits may explain ecological interactions in the subsystem where they occur and even, rather unintuitively, in the other subsystem (Bezemer and van Dam 2005, Kaplan et al. 2008). Remarkably, empirical evidence about the co-variation of multiple plant traits remains limited (Tjoelker et al. 2005, Ballhorn et al. 2013) and efforts to characterize variation in and ecological impacts of above- and belowground plant traits have proceeded somewhat independently. For example, variation in shoot chemical defenses is often evaluated in the context of how it impacts the preference and/or performance of folivorous insects (Harvey et al. 2011 and references therein). On

the other hand, until fairly recently (Rasmann and Agrawal 2008, Van Dam 2009) variation in root chemistry largely has been investigated in terms of its effects on plant competition (via allelopathy, Inderjit et al. 2011) and on root decomposition, relative to those of climate and environmental factors (Silver and Miya 2001, King et al. 2005). However, plants are integrated organisms whose responses to aboveground factors may in turn influence responses to belowground factors and *vice versa* (reviewed in Bardgett and Wardle 2010, Johnson et al. 2012).

It is tractable to consider plant-insect interactions as mediated by individual traits, e.g., shoot nitrogen content. In reality, though, plants express complex phenotypes comprised of multiple traits relating to growth, competition, defense, reproduction, etc. Recent studies have advocated for evaluating trait complexes (e.g., Ballhorn et al. 2013). In this sense, anti-herbivore defense, for example, can be thought of as a composite of traits relating to a plant's phenology, nutritional status, physical characteristics, direct and indirect defenses, and capacity to tolerate damage (Agrawal and Fishbein 2006). Similarly, ecophysiological functioning can be assessed as a suite (Agrawal et al. 2008), including carbon (C) : nitrogen (N) ratio, leaf water content, specific leaf area, trichome density, and water use efficiency (Woodman and Fernandes 1991, Garnier and Laurent 1994, Lambers et al. 1998, Meziane and Shipley 2001). In this study, we seek to advance our understanding by evaluating co-variation in multiple plant growth, defense, and ecophysiological traits and then testing for ecological impacts in an explicitly aboveground-belowground context.

Above- and belowground traits and trait complexes may be especially important to evaluate for plant species that are attacked by herbivores from different guilds. A

variety of herbivores utilize different tissues at different points in their ontogeny, e.g., adults feeding on leaves and larvae feeding on roots. Trait complexes encompassing the entire plant may be critical for understanding attack by even a single herbivore species if it has an above-belowground life cycle. Moreover, traits that mediate plant-herbivore interactions may differ between the two plant subsystems. For example, leaf hairs (trichomes) may deter foliar feeders, but cannot directly impact root feeders. Thus, understanding how plant shoot and root traits are correlated within individual plants and how these correlations vary among plant genotypes becomes important for predicting differences in the performance of above- and belowground insect life stages.

Interactions between common milkweed *Asclepias syriaca* L. and the red milkweed beetle *Tetraopes tetraophthalmus* Forster, a (co)evolved herbivore (Farrell and Mitter 1998), provide an excellent system to address these issues for three main reasons. First, in natural populations, the above- and belowground life stages of this insect use this plant as their sole resource. Because the two life stages employ different strategies to identify and utilize their host, this sets up the conditions that could produce trade-offs in host defense traits. Moreover, foliar-feeding adults are exposed to somewhat different plant phenotypes than root-feeding larvae: toxic cardenolides are present throughout the plant body, but latex and trichomes are found only in shoots (Malcolm 1991, Rasmann et al. 2011). Second, herbivory by larvae causes substantial damage to main roots (Rasmann et al. 2011, Erwin et al., *in press*), which may increase the mortality of young plants and/or those with small root systems. Thus, this herbivore is a potentially important agent of natural selection. Finally, as common milkweed is a model for understanding the ecological and evolutionary implications of genotypic

variation (Agrawal 2004, 2005, Bingham and Agrawal 2010, Abdala-Roberts et al. 2012), we already have detailed knowledge about plant traits that can mediate interactions with specialized insect herbivores, including *T. tetraophthalmus*.

In this study, we take an integrative whole plant / whole life cycle approach to reflect the nature of above-belowground interactions between common milkweed and the red milkweed beetle. We characterized the extent to which 14 plant traits are genetically correlated in expression, and asked how these traits affect the above- and belowground life stages of *T. tetraophthalmus*. Specifically, we determined (1) the variability and heritability of above- and belowground plant traits relating to growth, defense, and ecophysiology; (2) if traits and/or clusters (= syndromes) predict leaf herbivory, oviposition, larval emergence, root herbivory, and larval survival and mass; and (3) how adult and larval performance are correlated across plant genotypes.

METHODS

Natural history. Common milkweed is a perennial plant native to North America and found in disturbed areas and early successional habitats throughout the eastern United States (Wyatt 1996). In central New York, USA, ramets typically emerge in late May and flower from mid-June through July. The species persists through a combination of vegetative propagation via rhizomes and sexual reproduction via hermaphroditic flowers (Agrawal 2004). A single pollen sac (= pollinium) sires all seeds produced by a flower and thus fruits (= pods) contain full-sibling progeny (Gold and Shore 1995).

Common milkweed's well-characterized suite of aboveground defense traits (Agrawal 2004, 2005, Agrawal and Fishbein 2006) includes toxic cardenolides, sticky

latex, dense trichomes, tough leaves, and shoots that vary in C:N ratio. Most of these traits vary among naturally occurring milkweed genetic families (hereafter genotypes) and have been demonstrated to confer some benefit to the plant and/or reduce the performance of insect herbivores (e.g., Agrawal 2004, Van Zandt and Agrawal 2004, Agrawal and Fishbein 2006). Belowground, cardenolides are also present and variable among genotypes (Rasmann et al. 2009), although they have not always been shown to negatively impact the performance of belowground herbivores (A. Erwin et al., *in press*). As trichomes and latex are not present belowground, the performance of root-feeding insects is expected to be influenced by other plant traits such as root nutritional quality and the length and diameter of the main root (A. Erwin et al. *in press*).

The red milkweed beetle is a monophagous herbivore of common milkweed (Wyatt 1996), although adults occasionally are found on congeners (Matter 2001). In central New York, adult beetles emerge from the soil in late June, and feed and congregate on the young leaves of flowering stems (Matter et al. 1999, Reagel et al. 2002). Over the course of their ~30 day lifespan, females mate repeatedly and oviposit numerous clutches of ~12 eggs (Reagel et al. 2002). Despite the specialized diet of *T. tetraophthalmus*, females do not oviposit on their host plant but in the dry flowering stems of nearby grasses and forbs, such as *Festuca* and *Bromus* spp. (Gardiner 1961, Matter 2001, Agrawal 2004). Eggs hatch after 6 - 12 days, and larvae drop to the ground and feed on milkweed roots (Matter 2001), usually boring into third-order (larger diameter) 'main' roots and feeding less on first and second-order (smaller diameter) 'fine' roots (Erwin et al., *in press*).

Experimental set-up. Plants were grown in April 2012 using seeds collected in

October 2010 from four old fields across Tompkins Co., New York, USA. These fields (= populations) are each separated by approximately 8 km, are roughly the same size, and each have numerous long-lived patches (~ genets) of common milkweed. Patches were differentiated based on proximity and density of the ramets as well as several morphological traits (A. Erwin, *unpublished data*). In five patches per population, one mature fruit was selected from one ramet.

Seeds from each fruit were cold stratified at 4°C on moist filter paper for a week, nicked, and germinated in the dark at 26° C. Plastic pots (10 cm diameter) were filled with an equal volume of a 1:2 mixture of sand and a growing medium (Mix 111, Lambert Co., Rivière-Ouelle, Qc, Canada); sand was incorporated to improve pot drainage and facilitate fine root recovery. Seedlings were individually transplanted into pots and placed randomly in a growth chamber (14:10 hours D/N light, and 26:16 °C D/N temperature). Plants were watered daily, resulting in 50% ± 10% relative humidity in the chamber, and fertilized weekly with a dilute solution (N:P:K 21:5:20 150 ppm N (g/g)). Although we did not observe any chamber effects, we re-randomized plants four weeks after planting. After eight weeks in this common environment, plants were randomly assigned to one of three experiments: 1) plant trait variation, 2) adult assay, or 3) larval assay.

For Experiments 2 and 3, we used wild-caught adults and laboratory-reared larvae of *T. tetraophthalmus*. We collected adults from common milkweed patches across Tompkins Co., NY, sexed them, and kept mixed-sex groups comprised of 5 males and 5 females in ventilated holding containers (30 cm × 20 cm × 15 cm) under natural light at ambient humidity. We provided each group with fresh common

milkweed leaves (for food) each day. Usually, when in close proximity, adults mate within ten minutes (Gontijo 2013) and, indeed, we regularly observed mating events throughout the two days they were kept in this condition. After two days in mixed-sex groups (during which time we assumed each female had been mated), females were removed and kept in separate containers without food for ~ 24 hours. To make sure they were sexed correctly, adults in the female-only containers were observed intermittently to ensure that no mating occurred and any males were removed. Because we were testing for differences in leaf herbivory, oviposition, and larval emergence on different plant genotypes, starting with *mated females* was an important pre-condition.

Experiment 1: Genetic basis of plant trait variation. No treatments were imposed on plants assigned to Experiment 1 (total $n = 100$, 5 replicates in each of 20 genotypes) because we were interested in quantifying standing variation among the genotypes. Eight weeks after planting, we measured 14 above- and belowground traits that are potentially associated with host plant use by insects. Because insects may consume more and/or have higher performance on more vigorous plants, we measured stem height, total number of leaves, the length of the longest leaf, and total shoot dry mass as indices of aboveground growth; we measured main root length, main root diameter, and the mass of 'main' and 'fine' roots as indices of belowground growth. To assess variation in anti-herbivore defenses and plant quality, we measured foliar trichome density (Mauricio and Rausher 1997) and the C:N ratio and total concentration of cardenolides (e.g., Agrawal 2004, 2005, Rasmann et al. 2009) in both shoot and main root tissues. We also quantified the number of root buds (= points where daughter ramets grow out of a maternal plant's rhizome) as an index of clonality. Measurements

were made and tissues were prepared for C:N and cardenolide analysis following the standard methods of our laboratory, explained in detail elsewhere (Agrawal 2004 Appendix A, Agrawal 2005, Rasmann et al. 2011). To meet assumptions of normality, data were log transformed and outliers were excluded if they were larger or smaller than 1.5 times the inter-quartile range.

We report the range (across populations and genotypes), full-sibling heritability (Roff 1997), and full-sibling evolvability (Houle 1992, Agrawal et al. 2008) of these traits. Heritabilities were calculated as 2 × variance component of genotype/total variance; these values are typically lower than broad sense heritabilities, but approach narrow sense heritabilities when all genetic variance is additive (Roff 1997). We calculated the evolvability of each trait as 100 × (sqrt(genotype variance component × 2)/mean trait value) (Houle 1992).

To characterize integration at the individual plant level, we ran Pearson product-moment correlations (Agrawal et al. 2008) between all trait pairs. Because none of the pairwise correlations is statistically inevitable, our null hypothesis was that there would be no correlations between the traits (Agrawal et al. 2008). Nonetheless, overall plant vigor could easily explain positive correlations between some traits, such as above- and belowground plant size measures. Moreover, finding strong relationships (either positive or negative) between above- and belowground quality or defense traits would facilitate a strong preference-performance relationship between adult and larval beetles.

To evaluate whether genotypes form clusters on the basis of their traits, we used hierarchical cluster analysis (HCA; Becerra 1997, Agrawal and Fishbein 2006) to create a dendrogram from data standardized to Z-scores ((value-mean)/SD). Because the

length of the longest leaf and of the main root did not vary by population or genotype (Table 3.1), we excluded these two traits from the HCA, reducing our plant trait number from 14 to 12. We employed Ward's minimum variance method, in which the distance between two clusters is the ANOVA sum of squares between the two clusters summed over all the variables.

To determine how the 12 traits contributed to the phenotypic and/or population-based segregation of genotypes represented in the dendrogram, we conducted a principal component analysis on covariances with Varimax rotation. We tested for statistical support for clusters by performing a post-hoc MANOVA of all traits on cluster. Then we employed one-way ANOVA to test for whether cluster, principal components with eigenvalues over 1.0, or individual traits predict insect response variables (see Experiments 2 and 3, below). Data analyses were performed with the statistical software JMP, Version 10 (SAS Institute, Cary, NC, USA).

Experiment 2: Leaf herbivory, oviposition, and larval emergence on different plant genotypes. After being contained for ~24 hours without food or oviposition substrate, mated female beetles were individually added to bagged replicates of the same plant genotypes (n = 5 replicates * 20 genotypes = 100 plants and 100 beetles) that had been transported from the growth chamber to a large mesh cage in a local old field. To enable us to test for differences in oviposition during this no-choice assay, we placed in each pot a 15 cm long dried grass stem. Each day for five days, we unbagged the plant (being careful not to let the beetle escape) and quantified aboveground herbivory as the percent leaf area removed. At this time, we also removed the grass stem, counted

	Population Range of LSMs	Genotype Range of LSMs	Broad sense heritability	Evolvability
Trichome density (hairs/cm^2)	**248 - 410†**	**247 - 505****	0.530	31.04
Stem height (cm)	**10.49 - 19.32***	**12.70 - 19.90*****	0.799	20.88
Leaf no.	14.45 - 14.65	**13.53 - 16.22***	0.331	8.65
Log(Leaf length) (cm)	2.29 - 2.30	2.26 - 2.35	0.168	2.51
AG cardenolides (ug/mg dw)	**0.52 - 0.86****	0.52 - 0.86	0.008	4.09
AG C:N	11.24 - 11.90	**10.68 - 12.86****	0.447	10.65
Log(Total AG dry mass) (mg)	6.93 - 6.95	**6.72 - 7.40****	0.483	4.56
Log(Main root length) (cm)	2.57 - 5.84	2.56 - 2.91	0.085	6.08
Main root diameter (mm)	**4.53 - 5.36***	4.46 - 5.43	0.040	4.49
Log(Root bud no.)	2.99 - 3.07	**2.55 - 3.49*****	0.778	14.06
Log(Main root cardenolides) (ug/mg dw)	**0.44 - 0.60†**	**0.46 - 0.64****	0.511	18.43
Log(Main root C:N)	**3.74 - 3.86†**	3.73 - 3.88	0.078	1.81
Main root dry mass (mg)	**342.70 - 479.69***	314.91 - 547.16	0.178	19.20
Log(Fine root dry mass) (mg)	5.02 - 5.54	**4.98 - 5.82****	0.454	7.81
Leaf herbivory (cum. % area rmvd.)	177.60 - 251.33*	**143.20 - 276.23**	-0.105	.
Main root herbivory (length of dmg, cm)	13.96 - 14.71	**13.88 - 14.76**	0.004	3.39

Table 3.1 Upper: Range of variation, heritability, evolvability for 14 growth, defense, and ecophysiological traits of common milkweed *Asclepias syriaca.* Columns are calculated from genotypes growing in a common garden, with range of variation (least squared means, LSMs) reflecting the lowest to highest genotype means. *Notes:* The horizontal line in the middle of this section divides aboveground from belowground plant traits. AG: aboveground (= entire shoot); cardenolides: total concentration of cardenolides; C:N: carbon to nitrogen ratio. Lower: Range of variation, heritability, evolvability for leaf and main root herbivory on replicate plants of the same genotypes by adult and larval red milkweed beetles (*Tetraopes tetraophthalmus*). *Notes:* Evolvability of leaf herbivory could not be calculated because the variance component for genotype is negative. Because the distributions of other insect traits (oviposition marks, larval emergence, survival, and mass) did not approach normality, these traits were not tested and are not shown. Significance of population and genotype were determined with the likelihood ratio χ^2 test for random effects and levels are indicated as follows: † $P \leq 0.1$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.0001$; these values are also highlighted in boldface type.

the number of oviposition marks on it, and replaced it with a fresh grass stem (in case females can detect, and are deterred from ovipositing in, stems already containing eggs). We repeated this procedure each day for five days and then summed our measures of leaf herbivory and oviposition for each female on each plant. Because it is not known how many eggs are usually laid per oviposition mark, we labeled each stem, brought it back to the laboratory, and counted the number of larvae emerging over the course of a week. This procedure enabled us to test for a correlation between the number of oviposition marks and the number of emerging larvae; we expected it to be strongly positive. Of the three indices of host plant suitability measured in this no choice assay (leaf herbivory, oviposition marks, and emerging larvae) the last is perhaps the most closely tied to fitness.

Since our index of leaf herbivory was normal, genotypic least squared means were fit using a *glm* in the statistical program R (R Development Core Team, 2012). Oviposition and larval emergence, on the other hand, followed a negative binomial distribution and so we generated genotypic least squared means from a *glm.nb* in the same program.

Experiment 3: Root herbivory, larval survival and mass on different plant genotypes. Plants in Experiments 3 were used in a separate no choice assay to test for differences in the performance of *T. tetraophthalmus* larvae feeding on the same 20 genotypes. As in Experiment 2, we used a set of replicate plants that had been transported from the growth chamber to a large mesh cage in a local old field. Since we did not need to confine herbivores to plant shoots, the shoots of plants were not bagged in mesh.

From among the hundreds of neonates produced by female beetles used in Experiment 2, a subset of healthy (= wriggling, bright yellow) larvae were used in Experiment 3. First-instar larvae were kept without food on moist filter paper in petri dishes (10 cm diameter) for < 24 hrs before being transferred to experimental plants. To add them, we made a small hole (2.5 cm away from the plant stem, 1 cm deep) in the soil mixture with the end of a paintbrush, into which we gently placed the larvae. With 5 larvae per plant, 5 plants per genotype and 20 genotypes, this design yielded 100 plants and 500 larvae.

Ten days after applying larvae, we carefully loosened the soil mixture and collected surviving insects by hand. Insects were brushed to remove soil, counted, and weighed individually. Because initial weight was very consistent (0.133 ± 0.005 mg; n = 20) we did not weigh each individual larva before adding it to an experimental pot. Consequently, instead of calculating insect growth as final minus initial mass, we calculated it on a per pot basis as the total mass of surviving larvae divided by the number of surviving larvae. This metric allows us to test for any effects of genotype on mass without potentially confounding mass with survival. We calculated insect survival as the number of surviving larvae re-collected from a pot relative to the number that we added initially. We generated genotypic means for individual larval mass using a linear mixed-effects model (*lmer* function from the lme4 package for R) that accounted for plant as a random effect; to calculate genotypic means for larval survival, we used logistic regression on the number of larvae that did and did not survive (out of the 5 we initially added).

We then washed the roots and separated the main root from the fine roots using

a sterile blade. We quantified main root length with a ruler. Damage to the main root was visible to the unaided eye and characterized by direct consumption in the form of bore marks, i.e., 'tunnels' through an outer 'shell' of main root. This approach to quantifying damage—measuring individual sections of damaged tissue and summing over the entire root system—is considerably more detailed than other methods that are qualitative or based on a visual scale (Erwin et al. *in press*). Since root herbivory and larval mass data were roughly normally distributed, we generated genotypic means for these traits using a *glm* in R.

Finally, to characterize the relationship between adult and larval performance at the individual plant level we ran Spearman's non-parametric correlations on the genotypic means of all insect traits (i.e., leaf herbivory, oviposition marks, larval emergence, root herbivory, larval survival and mass). Back-transformed least squared means for insect response variables are shown in figures.

RESULTS

Out of the 14 plant traits we measured, seven varied significantly among natural populations and eight showed genotypic variation (i.e., significant full-sib heritability), although for only three traits (trichome density, stem height, and main root cardenolides) did we detect variation at both levels (Table 3.1). Two other traits (the length of the longest leaf and length of the main root) did not vary by population or genotype and were excluded from further analyses. Heritability values for plant traits were moderate overall, and very similar for above- and belowground traits (0.395 and 0.303, respectively). In contrast, heritability values for leaf and root herbivory by adult and larval beetles, respectively, were very close to zero (Table 3.1). The evolvability of

most plant traits was substantial; aboveground traits had slightly higher evolvability (11.768 on average) compared to belowground traits (10.269 on average). Evolvability of leaf herbivory could not be calculated because the variance component for genotype was negative. The evolvability of root herbivory (3.39) was lower than all but two of the plant traits. Because the other insect traits (oviposition, larval emergence, and larval mass) did not approach a normal distribution, we did not calculate heritability or evolvability values for them and they are not shown in Table 3.1.

We detected mostly positive pairwise correlations within and between plant subsystems. Out of the 91 correlations, 20 were significant and 7 were marginal (Table 3.2). The observed number of significant correlations is highly unlikely to have occurred by chance (binomial expansion test $P < 0.001$). Within the aboveground subsystem, all correlations were positive, with moderate to strong correlations between growth-related traits (i.e., stem height, leaf number, total mass; Table 3.2). Belowground, some growth-related traits were positively correlated (main root diameter and main root mass, main root mass and fine root mass), and we also detected correlations between different *types* of traits. Specifically, we observed strong correlations between main root length and main root cardenolides, and between main root C:N and fine root mass. The single negative correlation we detected—between main root length and main root cardenolides (Table 3.2, Fig. S3.1A)—was within the belowground subsystem, and implicates a trade-off between belowground growth and defense. The most striking cross-subsystem pattern was the correlation of aboveground C:N with every belowground trait except for main root length (Table 3.2), which, as noted above, did not vary at the population- or genotype level (Table 3.1).

	Trichome density	Stem height	Leaf no.	Log(Leaf length)	AG card-enolides	AG C:N	Log(Total AG dry mass)	Log(Main root length)	Main root diameter	Log(Root bud no.)	Log(Main root card-enolides)	Log(Main root C:N)	Main root dry mass
Trichome density (hairs/cm^2)													
Stem height (cm)	0.148												
Leaf no.	0.334	**0.767*** **											
Log(Leaf length) (cm)	-0.117	**0.399†**	0.323										
AG cardenolides (ug/mg dw)	-0.215	-0.023	-0.191	-0.321									
AG C:N	-0.039	**0.582** **	**0.449***	0.269	0.033								
Log(Total AG dry mass) (mg)	0.228	**0.609** **	**0.668** **	**0.502***	-0.157	**0.754*** **							
Log(Main root length) (cm)	-0.172	-0.098	-0.005	-0.057	0.063	0.048	-0.230						
Main root diameter (mm)	-0.159	0.224	-0.241	0.112	0.183	**0.437†**	0.283	-0.351					
Log(Root bud no.)	-0.177	0.179	0.224	0.337	-0.346	**0.484***	0.314	0.180	-0.081				
Log(Main root cardenolides)	-0.090	-0.226	-0.257	-0.171	0.129	**-0.389†**	-0.229	**-0.652** **	0.028	-0.290			
Log(Main root C:N)	**0.429†**	**0.503***	**0.386†**	0.157	0.190	**0.518***	**0.457***	-0.126	0.367	0.104	-0.259		
Main root dry mass (mg)	-0.193	0.269	-0.087	-0.053	**0.401†**	**0.675** **	0.317	-0.078	**0.785*** **	0.198	-0.041	**0.462***	
Log(Fine root dry mass) (mg)	0.244	**0.541***	**0.400†**	0.210	0.132	**0.767*** **	**0.702*** **	-0.248	0.353	0.330	-0.151	**0.677*** **	**0.582** **

Table 3.2 Pairwise correlations of 14 growth, defense, and ecophysiological traits of common milkweed *Asclepias syriaca. Notes*: Two of the correlations represent a trade-off, highlighted in grey. The horizontal line in the middle of the table divides aboveground from belowground plant traits. Abbreviations as in Table 3.1.

The hierarchical cluster analysis identified three groups (Fig. S3.2A), which we confirmed as being distinct using a post-hoc MANOVA (Appx. $F_{24, 12} = 4.29$, $p = 0.006$). A subsequent principal component analysis revealed that clusters were primarily characterized by having a small, cardenolide-rich main root, being large and poor quality (high C:N ratio), or having slightly higher foliar cardenolides and intermediate values of the aforementioned traits (Fig. S3.2B and C). Aboveground C:N ratio appears to be particularly important in distinguishing genotypes, as indicated by relatively long loading vectors in each panel of Fig. S3.2B. In contrast, the three vectors for root cardenolides shown in Fig. S3.2B are relatively short; we interpret this pattern to mean that root cardenolide concentration contributes relatively little to the differentiation of genotypes among clusters.

Univariate ANOVAs revealed that some traits (but not cluster) predicted measures of the performance of *T. tetraophthalmus* adults and larvae (Table S3.1). We found marginally significant (negative) effects of aboveground C:N as well as four belowground traits and Component 1 (which is based on the same traits, Table S3.2) on leaf herbivory by adults (Table S3.1). Additionally, we detected a significant (positive) effect of main root diameter, and a marginal (negative) effect of foliar cardenolides, on larval emergence (Table S3.1). These effects suggest that adult females prefer to lay fewer eggs after feeding on cardenolide-rich leaves, and more eggs following damage to plants with thick main roots. That larval emergence is not correlated with larval performance measures (i.e., root herbivory, larval survival and mass; Table 3.3) strongly suggests, however, that putative preferences of adult females do not translate to an increase in offspring fitness.

	Leaf herbivory	Oviposition marks	Larval emergence	Main root herbivory	Larval survival
Leaf herbivory					
Oviposition marks	**0.386†**				
Larval emergence	0.016	0.259			
Main root herbivory	0.306	-0.094	0.263		
Larval survival	0.224	-0.077	0.088	**0.648****	
Larval mass	0.227	**0.490***	0.059	-0.224	0.074

Table 3.3 Spearman's non-parametric correlations of six performance-related traits of the red milkweed beetle *Tetraopes tetraophthalmus.*

Additionally, we detected a marginally significant negative correlation between aboveground C:N and main root cardenolides, indicating that plant genotypes that could be relatively good hosts for adults would likely not be suitable for larvae and *vice versa* (at least based on these two traits). However, the two genotypes that seemed most suitable (lower C:N and low main root cardenolides; Fig. S3.3A), had average—not higher—levels of leaf and main root damage (Figs. S3.3B and C).

We did not detect many significant correlations between measures of adult and larval performance: out of the 15 correlations, two were significant and one was marginal; all of these were positive (Table 3.3). The observed number of significant correlations could have occurred by chance (binomial expansion test $P = 0.135$). We found a marginally significant (positive) correlation between leaf herbivory and oviposition marks (Table 3.3), suggesting that leaf phenotype (perhaps determined by foliar cardenolides, see above) influences oviposition behavior. The number of oviposition marks made by females was not correlated with the number of emerging larvae, in contrast to our expectation, but was significantly (positively) correlated with individual larval mass (Table 3.3, Fig. S3.1B). Main root herbivory was correlated positively with larval survival, as expected, but not with larval mass (Table 3.3).

DISCUSSION

Here we took a 'whole plant / whole life cycle' approach by characterizing above- and belowground trait variation in *Asclepias syriaca*, and then asking how single and integrative measures of plant variation affect the performance of *Tetraopes tetraophthalmus,* a specialist herbivore with a life cycle that crosses the 'soil barrier'.

Our results indicate that significant genotypic variation exists both above- and belowground, and that genotypes form distinct clusters (potential syndromes) based on both shoot and root traits. Yet, the variation and integration of traits that we detected did not translate to clear differences in the performance of shoot-feeding adults or root-feeding larvae. Finally, although we detected some evidence for adult host plant preference, this did not appear to be correlated with larval performance.

Plant trait variation and genotypic correlations. We have demonstrated standing variation at the population and/or genotype levels for 12 (out of 14) traits of *A. syriaca* relating to above- and belowground growth, defense, and ecophysiology. Such variation, in combination with moderate heritability and evolvability values, suggests that there is strong potential for selection on both the above- and belowground phenotype of this plant. Selection on a subset of these traits has been demonstrated in response to foliar herbivory by monarch caterpillars, drought stress, and nutrient enrichment (Agrawal et al. 2008). Nonetheless, numerous significant positive correlations within and between the subsystems indicates that a plant should be thought of as an integrated whole, rather than a pair of separate shoot and root phenotypes.

Genetic characteristics of plants may be influenced by the diversity of attacking herbivores, potentially resulting in or affecting existing correlations between traits (Hodges et al. 2013). Based on allocation costs and redundancy, we would expect a *negative* correlation between above- and belowground investment in a defense trait that is present in both subsystems. Hypothetically, if a large investment in cardenolides in shoots (the subsystem that first receives damage during the growing

season) is necessary and sufficient to deter adult beetles, then investment in root cardenolides (to resist subsequent larvae) could be small. Nonetheless, a strong *positive* correlation between investment in above- and belowground traits could be favored if selection by beetles was strong enough, and if neither defense mechanism was foolproof (Rasmann and Agrawal 2009).

In this study, a third (n = 7) of all pairwise correlations between aboveground traits (n = 21) were significant, and all of these were positive. Between belowground traits, fewer (5 out of 21) were significant, and one of these was negative, indicating a trade-off between belowground growth and defense as measured by main root length and main root cardenolides. Of the eight (out of 49) significant shoot-root correlations, half involved aboveground C:N ratio, suggesting an important role for this trait in defining the whole plant phenotype. Foliar C:N (or nitrogen content alone) has been previously shown in this system to have a genetic basis (e.g., Agrawal 2005, Agrawal et al. 2008).

The significant shoot-root correlations were mostly between different types of traits (e.g., aboveground C:N ratio and fine root biomass), rather than above- and belowground versions of the same trait (e.g., shoot and root cardenolides). Indeed, shoot and root cardenolides were not significantly genetically correlated. Our result at the intra-specific level is in contrast to an inter-specific comparison of 32 *Asclepias* species, which reported a positive correlation between root and shoot cardenolides, even after correcting for phylogenetic relatedness among species (Rasmann et al. 2009).

Interestingly, we detected a marginally significant negative correlation

between aboveground C:N and main root cardenolides, indicating that plant genotypes that could be relatively good hosts for adults would likely not be suitable for larvae and *vice versa*. The two genotypes that seemed most suitable (due to having lower C:N and lower main root cardenolides; Fig. S3A) did not have higher levels of leaf and main root damage (Figs. S3B and C), indicating that other traits or mechanisms likely influence feeding. Together, these results suggest that the aboveground phenotype an adult female is exposed to may have little predictive power for the belowground phenotype her larvae are likely to feed on. If so, we would expect natural selection for females to feed on plants that maximize their own individual fitness rather than plants that might be the most suitable hosts for their offspring (Scheirs et al. 2000).

Integrative measures of plant trait variation: syndromes. Consistent correlations between traits relating to leaf morphology, metabolism, and longevity have been demonstrated for diverse taxa and biomes, and are therefore thought to comprise leaf syndromes (Reich et al. 1999, Wright et al. 2004). For example, high rates of CO_2 exchange are associated with high concentrations of leaf nitrogen, high specific leaf area, and short lifespan across species (Tjoelker et al. 2005). Belowground, root longevity across species is negatively correlated with nitrogen concentration, specific respiration rates, and specific root length (Eissenstat et al. 2000) or root tissue density (Ryser 1996, Craine et al., 2001). Within a species, finest roots tend to have a higher nitrogen concentration, higher specific respiration rates, higher specific root lengths, and shorter lifespans than coarser roots (Pregitzer et al. 1997, 1998, Eissenstat et al. 2000, Anderson et al. 2003).

Considering cross-subsystem patterns, nitrogen concentration and tissue density of leaves were correlated with those of fine roots in 24 grass species (Craine and Lee 2003). Another study also reported concordance in above- and belowground traits: 39 grassland and savannah species exhibited similar rankings of leaf and root nitrogen, respiration rate, and longevity (Tjoelker et al. 2005). In general, though, few existing datasets enable us to compare numerous above- and belowground plant traits on the same set of plants (Tjoelker et al. 2005) and the correspondence of root traits with aboveground trait syndromes is not widely understood (Eissenstat et al. 2000, Bouma et al. 2001, Craine and Lee 2003), especially at the population and genotypic levels. The correspondence of leaf and root traits raises the possibility that root traits may, in part, be predicted from leaf traits, with obvious benefits for aboveground insects who seek to determine host plant quality for their later-feeding belowground offspring.

Our findings indicate that the significant correlations among shoot traits of 20 common milkweed genotypes are positive, and those among root traits of the same plants are all positive save one (the correlation between main root length and main root cardenolide concentration). Across subsystems, we found mostly positive correlations as well and half of these included foliar C:N ratio, which has been shown in this and other systems to be an important trait in defining plant phenotype and associated ecological interactions with herbivores (Reich et al. 1998, Tjoelker et al. 2005, Agrawal and Fishbein 2006).

These within- and between-subsystem correlations were used to classify genotypes into three clusters (potential syndromes) that are primarily characterized by

having a small, cardenolide-rich main root, being large and poor quality (high C:N ratio), or having slightly higher foliar cardenolides and intermediate values of the aforementioned traits. Agrawal and Fishbein (2006) showed that 24 species of *Asclepias* could be classified into three syndromes based on variation in aboveground defense traits. In that study, the syndromes were defines as follows: (A) low foliar C:N coupled with high trichomes and latex, (B) very high foliar C:N ratio, coupled with tough leaves and low water content, and (C) foliar low C:N and specific leaf area coupled with high leaf cardenolides. Although the traits that these authors measured is somewhat different than those measured in the present study, there are similar themes. Most notably, Agrawal and Fishbein (2006) showed at the species level – as we have shown here at the genotype level – that aboveground C:N is correlated with several other traits and plays an important role in distinguishing plants based on their phenotype.

Impacts of plant trait variation on adult and larval performance. Variation in plant traits can strongly impact herbivore preference for and performance on different hosts. Some of the reported variation is at the genotype level, with full- or half-sib families showing quantitative or qualitative variation in traits that attract or deter insect herbivores (e.g., Stinchcombe and Rausher 2001, Tiffin 2002, Agrawal and Van Zandt 2003).

For common milkweed, we have extensive knowledge about genotypic variation and the consequences of such variation for individuals, populations, and communities of associated (specialized) insect herbivores. Agrawal (2004) showed that growth, reproduction, and several resistance traits varied among 23 genotypes.

The abundance of red milkweed beetle adults was negatively genetically correlated with leaf trichome density and nitrogen content, and positively correlated with plant height in the field. Agrawal (2005) also reported that five defense-related traits (aboveground cardenolide concentration, latex exudation, trichome density, leaf toughness, and nitrogen content) were highly variable among genotypes, and that trichomes and latex production were each negatively genetically correlated with the abundances of five species of specialist insect herbivores.

In the present study, we assessed the impact of traits and trait clusters on the performance of both adult and larval life stages of a specialist herbivore. Our results indicate no strong, overall effect of above- or belowground traits and/or clusters on measures of adult or larval performance. The one exception to this general conclusion is that larval emergence was significantly higher when females had fed on plants with larger main roots. Since larvae were emerging from grass stems added to plants on which females had been feeding in a no choice assay (and not emerging from the host plants themselves) this correlation is somewhat surprising. Indeed, it is an intriguing possibility that females can perceive the belowground phenotype of plants whose leaves they eat. Our experiment did not enable us to uncover a mechanism for this effect but, because *T. tetraophthalmus* larvae preferentially feed on main and not fine roots of *A. syriaca* (Erwin et al. *in press*), this behavior could well be adaptive. This speculation about *T. tetraophthalmus* is in contrast to a demonstrated pattern of behavior by maternal vine weevils (*Otiorhynchus sulcatus* F.) which tend to lay eggs on red raspberry (*Rubus idaeus* cv. Glen Ample) plants with *smaller* root systems, a behavior likely to negatively affect offspring performance (Clark et al. 2011).

The preference – performance relationship in an above-belowground context. The preference-performance hypothesis (PPH) states that female insects will preferentially oviposit on plants that maximize the survival and performance of their larvae (Jaenike 1978). The hypothesis predicts that there should be a positive correlation between oviposition preference and offspring performance, and may be especially important for species whose larvae have little or no ability to choose a host (because they are immobile). Although this hypothesis could equally be applied to species that have above-belowground life cycles (Johnson et al. 2006), to date, it has mostly been investigated in species with completely aboveground life cycles (Clark et al. 2011). Data from the present study enable us to consider the PPH in an aboveground-belowground context, as Clark et al. (2011) have done recently. Although we detected a positive correlation between oviposition marks and larval mass, leaf herbivory and oviposition marks were uncorrelated with root damage by and the survival of larvae, indicating that putative oviposition preferences of adults (discussed above) did not improve larval performance overall. This pattern is inconsistent with the results of a separate experiment (Erwin et al., *unpublished data*) that found that larval survival was higher on plants that had previously been damaged aboveground by adults. Thus, we conclude that the ways in which adult beetles interact with plant shoots do not appear to consistently positively influence the performance of their offspring. Weak and nonexistent correlations between adult preference and larval performance have been demonstrated in other systems (e.g., Rausher 1979, Scheirs et al. 2004, Digweed 2006, Gripenberg et al. 2007, Clark et al. 2011), in contrast to theory that this link should be strong (e.g., Jaenike 1978) and

several studies showing that it is so in some cases (e.g., Craig et al. 1989, Heisswold et al. 2005).

Conclusions. Investigating the composition and ecological impacts of multivariate phenotypes is a major focus in evolutionary ecology (Pigliucci, 2004). In this study, our main goal was to ask if the performance of specialist shoot-feeding adults and root-feeding larvae of the red milkweed beetle are affected by plant genotypic variation and, if so, what traits mediate these effects. We found that genotypes varied significantly in the range, heritability, and evolvability of their shoot and root traits, and that these traits clustered genotypes into three potential syndromes. However, overall, numerous measures of adult and larval performance did not strongly correlate with our single (trait) and integrative (cluster, principal component) measures of plant variation, indicating a weak preference-performance relationship for this herbivore, at least at the level of plant genotype.

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APPENDIX:

SUPPLEMENTARY INFORMATION

CHAPTER ONE

The following are supplementary tables and figures.

	Insect treatment			
	Tetraopes only		*Tetraopes* + Wireworms	
Insect density	Low N	High N	Low N	High N
4	8.7	5.1	5.74	6.32
5	6	6	n/a	n/a
6	6.75	9.4	14.73	13.23

Table S1.1 Values (cm) substituted for mean main root damage when no main root was recovered. N refers to nutrient level.

	Plant Damage		Plant Biomass		
Factor	MR	FR	SH	MR	FR
Identity					
Wireworms	3.96 ± 0.60	3.75 ± 0.43	5.02 ± 0.08	4.99 ± 0.14	4.08 ± 0.09
T. tetraophthalmus	6.31 ± 0.63	0.49 ± 0.44	4.38 ± 0.08	1.78 ± 0.14	3.01 ± 0.09
Nutrients					
Low	4.55 ± 0.62	1.67 ± 0.44	4.08 ± 0.08	3.14 ± 0.14	3.29 ± 0.09
High	5.72 ± 0.62	2.57 ± 0.43	5.31 ± 0.08	3.63 ± 0.14	3.80 ± 0.09
Identity*Nutrients					
Wireworms * Low	3.92 ± 0.86	2.98 ± 0.61	4.32 ± 0.11	4.55 ± 0.19	3.65 ± 0.12
Wireworms * High	4.01 ± 0.85	4.52 ± 0.60	5.71 ± 0.11	5.43 ± 0.19	4.52 ± 0.12
T. tetraophthalmus * Low	5.17 ± 0.89	0.35 ± 0.62	3.84 ± 0.11	1.73 ± 0.19	2.93 ± 0.13
T. tetraophthalmus * High	7.44 ± 0.91	0.62 ± 0.62	4.91 ± 0.11	1.83 ± 0.19	3.08 ± 0.13

Table S1.2 Least squares means ± standard errors of *Asclepias syriaca* main root (MR) and fine root (FR) damage (cm), and shoot (SH), MR, and FR biomass (mg) as predicted by insect identity, soil nutrient level, and their interaction. The Identity term indicates which insect was present.

	Insect Mass	
Factor	Wireworms	*T. tetraophthalmus*
Identity		
With conspecifics only	20.40 ± 0.94	19.69 ± 2.20
With con- and heterospecifics	19.41 ± 1.59	23.67 ± 3.96
Nutrients		
Low	21.95 ± 1.24	13.81 ± 3.35
High	17.86 ± 1.36	29.55 ± 3.06
Identity*Nutrients		
With conspecifics only * Low	21.50 ± 1.35	17.92 ± 3.23
With conspecifics only * High	19.31 ± 1.31	21.47 ± 2.99
With con- and heterospecifics * Low	22.41 ± 2.09	9.71 ± 5.86
With con- and heterospecifics * High	16.41 ± 2.39	37.63 ± 5.33

Table S1.3 Least squares means ± standard errors of the mass of individual wireworms and *Tetraopes tetraophthalmus* larvae (mg) as predicted by insect identity, soil nutrient level, and their interaction. The Identity term refers to whether heterospecific insects were present.

Factor	SH $_{(1, 77)}$	MR $_{(1, 77)}$	FR $_{(1, 77)}$
Density	0.01	0.30	0.14
Nutrients	**86.2076*** **	**23.0697*** **	**27.5047*** **
Density*Nutrients	0.48	0.19	0.00

Table S1.4 Analysis of covariance (ANCOVA) of wireworm density, soil nutrient level, and their interaction as predictors of *Asclepias syriaca* shoot (SH), main root (MR), and fine root (FR) biomass (mg). By excluding *Tetraopes tetraophthalmus* data, this analysis tests whether the significant effects of density, identity-by-density, and identity-by-nutrients in the whole model (Table 1.1) were driven by *T. tetraophthalmus* alone. Numbers in parentheses after column headers refer to df. Numbers in table are F-values with asterisks indicating significant differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Parameters	Model ID	*k*	AICc	ΔAICc	w_i	LL
(a) *H. abbreviatus*						
Dens, Hetero	3	3	**289.15**	0.00	0.51	-141.47
Dens, Hetero, Nutr	9	4	291.09	1.94	0.20	-141.37
Dens, Hetero, Dens * Hetero	6	4	291.26	2.10	0.18	-141.46
Hetero	2	2	293.16	4.01	0.07	-144.53
Hetero, Nutr	5	3	295.05	5.90	0.03	-144.42
Hetero, Nutr, Hetero * Nutr	8	4	296.60	7.45	0.01	-144.13
Dens	1	2	300.90	11.75	0.00	-148.40
Dens, Nutr	4	3	302.80	13.65	0.00	-148.30
Dens, Nutr, Dens * Nutr	7	4	304.85	15.70	0.00	-148.26
(b) *T. tetraophthalmus*						
Dens	1	2	**172.98**	0.00	0.28	-84.45
Dens, Hetero	3	3	173.72	0.74	0.19	-83.77
Dens, Nutr	4	3	173.72	0.74	0.19	-83.77
Dens, Hetero, Nutr	9	4	174.49	1.51	0.13	-83.09
Dens, Hetero, Dens * Hetero	6	4	174.75	1.77	0.11	-83.22
Dens, Nutr, Dens * Nutr	7	4	175.01	2.03	0.10	-83.35
Hetero	2	2	190.56	17.58	0.00	-93.23
Hetero, Nutr	5	3	191.19	18.21	0.00	-92.51
Hetero, Nutr, Hetero * Nutr	8	4	193.18	20.20	0.00	-92.44

Table S1.5 Model selection statistics to estimate survival of wireworms and *Tetraopes tetraophthalmus* larvae. Dens, insect density; Hetero, presence of heterospecifics; Nutr, soil nutrient level; *k*, number of parameters in a model; ΔAICc, difference between an alternative model and the best model; w_i, weight, indicating relative likelihood of model *i*; LL, log likelihood value.

Parameter	Coefficient	SE	Lower CI	Upper CI	Relative Importance
Wireworm survival					
Intercept	-0.87	0.50	-1.85	0.11	.
Dens	-0.18	0.09	**-0.35**	**-0.01**	0.89
Hetero	0.86	0.39	**0.09**	**1.62**	1.00
Nutr	-0.08	0.24	-0.56	0.39	0.24
Dens*Hetero	0.03	0.16	-0.28	0.34	0.18
Dens*Nutr	-0.04	0.14	-0.32	0.23	0.00
Hetero*Nutr	-0.37	0.49	-1.33	0.59	0.01
T. tetraophthalmus survival					
Intercept	-0.53	0.64	-1.80	0.73	.
Dens	-0.45	0.14	**-0.73**	**-0.17**	1.00
Hetero	0.67	0.73	-0.77	2.12	0.43
Nutr	-0.22	0.6	-1.41	0.96	0.42
Dens*Hetero	-0.25	0.24	-0.73	0.22	0.11
Dens*Nutr	-0.21	0.23	-0.65	0.24	0.10
Hetero*Nutr	0.27	0.76	-1.22	1.77	0.00

Table S1.6 Model-averaged coefficients and associated statistics for parameters predicting the survival of wireworms and *Tetraopes tetraophthalmus* larvae. Dens, insect density; Hetero, presence of heterospecifics; Nutr, soil nutrient level. Confidence intervals that exclude zero indicate that that parameter is a key predictor of survival. Relative importance values provide a relative metric of statistical support for the impact of each parameter.

Element	Percentage
Fertilizer	
Total nitrogen (N)	21.00
Ammoniacal	7.92
Nitrate	13.08
Available phosphate (P2O5)	5.00
Soluble potash (K2O)	20.00
Magnesium (Mg), water soluble	0.15
Zinc (Zn), chelated	0.0525
Manganese (Mn), chelated	0.0525
Boron (B)	0.0210
Copper (Cu), chelated	0.0105
Iron (Fe), chelated	0.0105
Molybdenum (Mo)	0.0105
Epsom salts	
MgSO4, water soluble	0.0400

Table S1.7 Composition of the solution—made of Jack's Professional LX All Purpose Water Soluble Fertilizer (J. R. Peters, Inc. Allentown, PA, USA) plus EPSO Top® Epsom salt (K+S KALI GmbH, Kassel, Germany)—used to create the 'high' and 'low' soil fertility treatments. Elements are derived from ammonium nitrate, potassium phosphate, potassium nitrate, magnesium sulfate, boric acid, iron EDTA, manganese EDTA, zinc EDTA, copper EDTA, and ammonium molybdate.

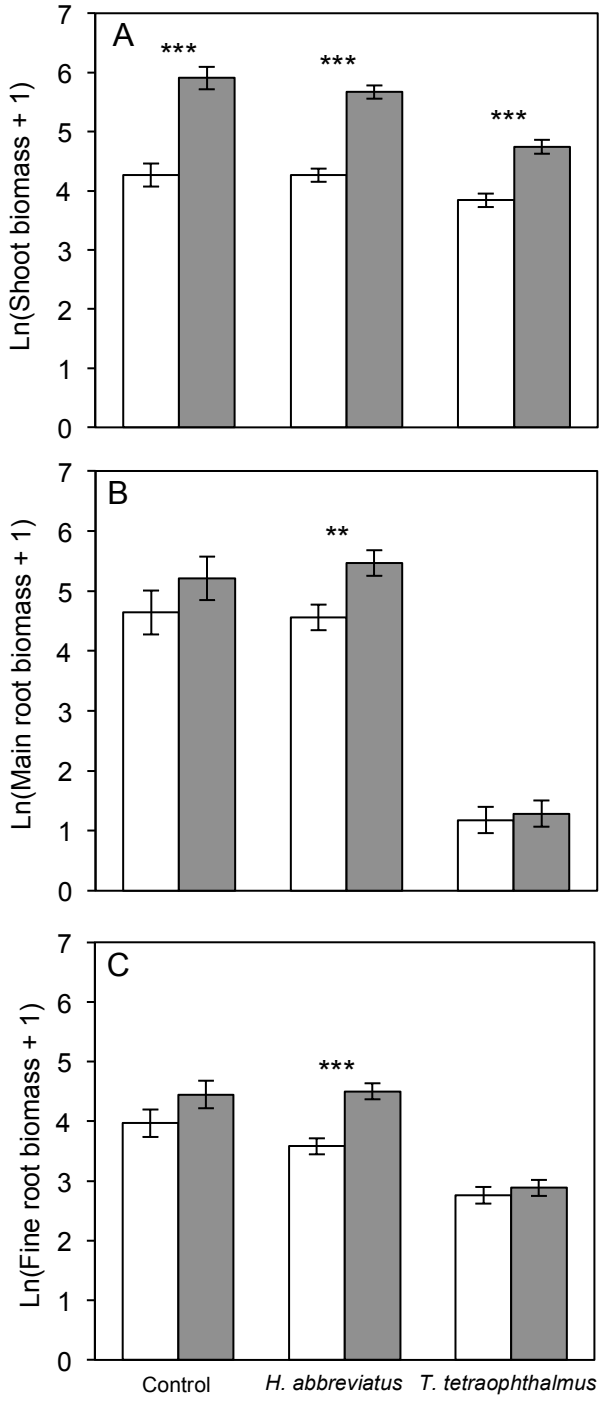

Figure S1.1 Biomass of plant shoots (A), main roots (B), and fine roots (C) growing in low (white bars) and high (grey bars) nutrient soil when no insects were present (control) or when either wireworms or *Tetraopes tetraophthalmus* larvae were present. Shoot biomass C: $F_{1, 180} = 36.38$; H: $F_{1, 180} = 78.88$; T: $F_{1, 180} = 30.21$. Main root biomass C: $F_{1, 181} = 1.24$; H: $F_{1, 181} = 8.90$; T: $F_{1, 181} = 0.12$. Fine root biomass C: $F_{1, 181} = 2.16$; H: $F_{1, 181} = 23.09$; T: $F_{1, 181} = 0.42$. Asterisks indicate significant differences

at $P \leq 0.05$ (*), $P \leq 0.01$ (**) and $P \leq 0.001$ (***)

CHAPTER TWO

The following are supplementary tables and figures.

Model	*Tetraopes*	*Danaus* (Wk 1-4)	*Labidomera* (Wk 5-8)
Abv + Blw + Week + Lvs + AbvxBlw + AbvxWeek + BlwxWeek + AbvxBlwxWeek	df = 10, AIC = 345.06	df = 10, AIC = 206.96	df = 10, AIC = 883.45
Abv + Blw + Week + Lvs + AbvxWeek + BlwxWeek	**df = 8, AIC = 341.12**	df = 8, AIC = 204.71	df = 8, AIC = 879.44
Abv + Blw + Week + Lvs + AbvxWeek	df = 7, AIC = 343.40	df = 7, AIC = 202.93	df = 7, AIC = 877.85
Abv + Blw + Week + Lvs + BlwxWeek	df = 7, AIC = 347.46	df = 7, AIC = 204.22	df = 8, AIC = 877.84
Abv + Blw + Week + Lvs	df = 6, AIC = 350.13	df = 6, AIC = 202.46	df = 6, AIC = 878.38
Abv + Week + Lvs	df = 5, AIC = 348.14	**df = 5, AIC = 200.46**	df = 5, AIC = 875.19
Blw + Week + Lvs	df = 5, AIC = 376.46	df = 5, AIC = 207.04	df = 5, AIC = 881.19
Alw + Lvs	df = 4, AIC = 355.02	df = 4, AIC = 230.19	**df = 4, AIC = 876.73**
Alw + Week	df = 4, AIC = 367.51	df = 4, AIC = 202.85	df = 4, AIC = 895.93
Week + Lvs	df = 4, AIC = 374.49	df = 4, AIC = 205.04	df = 4, AIC = 880.17
Week			df = 3, AIC = 899.54
Abv			df = 3, AIC = 894.70
Lvs			df = 3, AIC = 881.48

Table S2.1 Results of model selection using Akaike's information criterion (AIC) to identify terms with a significant effect on later-season leaf damage to *Asclepias syriaca* field plants for models with non-normal data. Models are for proportion of damaged plants by *Tetraopes tetraophthalmus* and *Danaus plexippus* (binomial), and for number of damage marks by *Labidomera clivicollis* (poisson). For each herbivore, a subset of all possible nested models is shown. Abv: initial aboveground herbivory by *T tetraophthalmus* adults; Blw: initial belowground herbivory by *T. tetraophthalmus* larvae; Lvs: total leaf number. . For *L. clivicollis* only weeks 5 to 8 are analyzed as virtually no damage was observed in the first half of the experiment. In contrast, only weeks 1 to 4 were analyzed for *D. plexippus* because at later times all plants had some leaf damage.

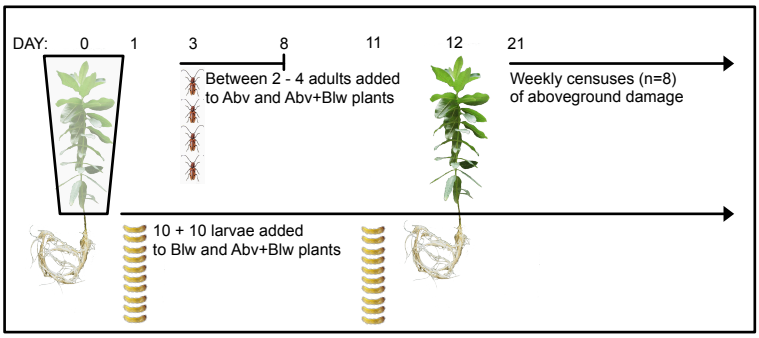

Figure S2.1 Timeline of the field experiment. All plants were bagged to exclude non-treatment herbivores on Day 0 (30 Jun. 2011). To mimic the timing of egg-laying by adult females and impose approximately 10 % damage to main roots (Erwin et al. 2013), plants in the Belowground (Blw) and Above- plus Belowground (Abv+Blw) herbivory treatments received 10 *Tetraopes tetraophthalmus* larvae on Day 1 and another set of 10 larvae on Day 11. That we did not terminate this treatment (by re-collecting larvae from plant roots) is indicated by a right-facing arrow. To impose the same amount of damage (~10 %) to leaves, we exposed plants in the Aboveground (Abv) and Abv+Blw herbivory treatments to *Tetraopes tetraophthalmus* adults during Days 3-8. Because of natural variation in plant defenses, different numbers of adults (2-4) were added to achieve this amount of damage. That we experimentally ended this treatment (by re-collecting adults from bags) is indicated by a line cap, rather than an arrow. Plants in the Control treatment were bagged and undamaged aboveground. On Day 12, bags were removed from all plants. Censuses of leaf damage commenced on Day 21 (21 Jul. 2011) and continued for eight weeks, until 9 Sep. 2012, when plants began to senesce.

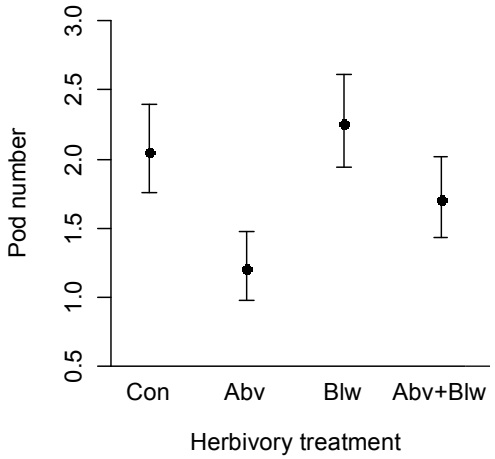

Figure S2.2 Impact of initial aboveground herbivory by adult, and belowground herbivory by larval, *Tetraopes tetraophthalmus* on the number of fruits produced by *Asclepias syriaca* field plants. Treatments consist of Con: undamaged control; Abv: aboveground herbivory only; Blw: belowground herbivory only; Abv+Blw: above- and belowground herbivory. Values are ± 1 SE. N = 20 plants per treatment.

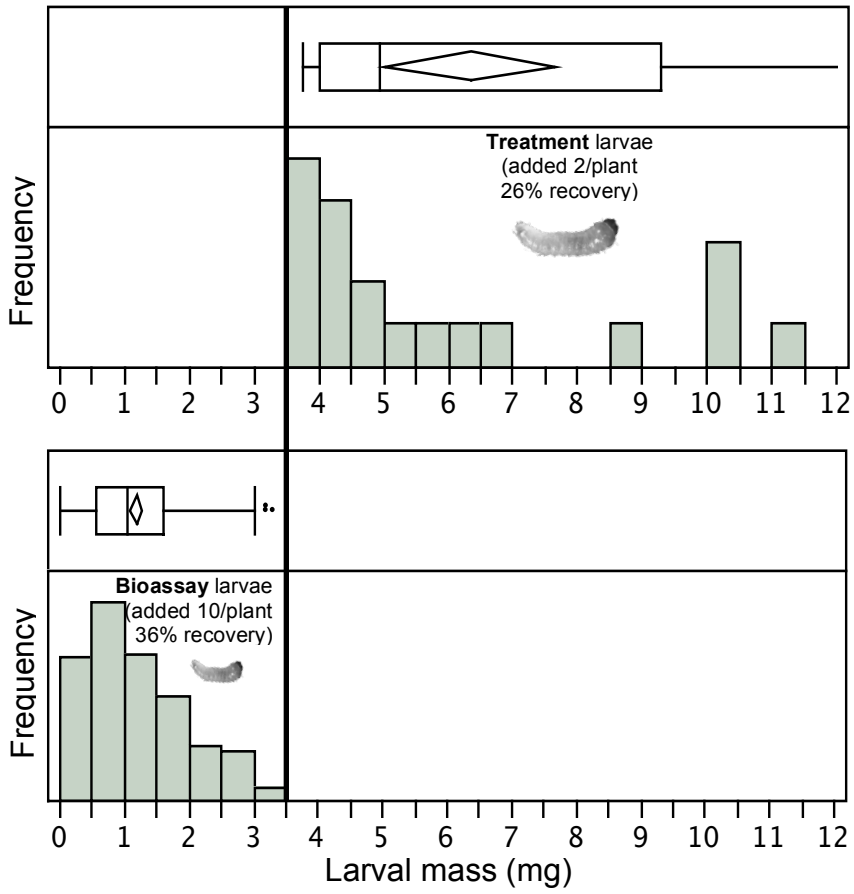

Figure S2.3 We were able to distinguish between larvae that were applied first (to impose belowground herbivory) and larvae that were applied eight days later (for the bioassay) based on visual observations during the harvest. Treatment larvae were lighter in color and large (6.34 ± 0.17 mg, N=21) whereas bioassay larvae were dark yellow and had a mean mass (1.17 ± 0.17 mg, N=280) similar to that of neonates. We assigned a post-hoc cut-off of 3.77 mg to divide the two groups. The largest treatment larvae were found in root systems with the largest bioassay larvae and vice versa (rather than all larvae being close to the cut-off value), suggesting that variation in plant vigor may also influence larval performance. Histogram of individual larval masses. Larvae applied first and used to impose belowground herbivory (treatment larvae) are shown on the upper panel. Larvae applied eight days later, for the bioassay, are shown in the lower panel. The bold vertical line represents the cut-off (3.77 mg) calculated after group assignment based on visual estimation during the harvest (see Results).

CHAPTER THREE

The following are supplementary tables and figures.

	Leaf herbivory	Oviposition marks	Larval emergence	Main root herbivory	Larval survival	Larval mass
INTEGRATIVE MEASURES						
Cluster	0.69	1.78	0.28	1.38	0.22	0.71
Component 1	**3.30†**	0.10	0.06	0.42	0.03	0.95
Component 2	0.19	0.62	0.20	0.29	0.71	0.57
Component 3	0.65	2.46	1.83	0.36	0.41	0.00
INDIVIDUAL MEASURES						
Trichome density	0.07	1.10	0.77	2.58	2.68	0.50
Stem height	0.05	0.36	0.01	0.03	0.10	2.11
Leaf no.	0.06	0.37	0.22	0.24	0.22	1.45
AG cardenolides	0.73	0.25	**3.05†**	0.05	0.63	0.51
AG C:N	**4.29†**	0.20	0.11	0.57	0.03	0.11
Log(Total AG dry mass)	0.60	1.16	0.94	1.26	0.60	1.28
Main root diameter	1.12	0.47	**6.22***	0.09	0.01	0.13
Log(Root bud no.)	**3.74†**	1.38	0.01	0.33	0.31	1.59
Log(Main root cardenolides)	0.03	0.24	0.99	2.92	0.00	0.24
Log(Main root C:N)	**4.26†**	1.11	0.13	0.26	0.47	0.03
Main root dry mass	**3.34†**	0.62	0.22	0.03	0.24	0.00
Log(Fine root dry mass)	**3.66†**	0.39	0.69	0.00	0.01	0.69

Table S3.1 ANOVA of cluster (from hierarchical cluster analysis), components (from principal component analysis) and 12 growth, defense, and ecophysiological traits of common milkweed *Asclepias syriaca* on measures of the performance of adult and larval *Tetraopes tetraophthalmus* beetles. *Notes*: Length of the longest leaf and of the main root were not included because we did not detect variation at the population or family level for these two traits (see Table 3.1). df = (2, 17) for cluster and (1, 18) for all other predictors. The horizontal line in the middle of the table divides aboveground from belowground plant traits. Abbreviations as in Table 3.1.

	Variable code	Component 1	Component 2	Component 3
Eigenvalue		4.689	2.309	1.479
Percent		39.073	19.240	12.321
Cum. Percent		39.073	58.313	70.633
Trait loadings				
Trichome density	1	0.208	-0.475	0.649
Stem height	2	0.758	-0.227	0.128
Leaf no.	3	0.613	-0.637	0.139
AG cardenolides	4	0.033	0.629	0.366
AG C:N	5	0.905	0.118	-0.273
Log(Total AG dry mass)	6	0.834	-0.216	-0.024
Main root diameter	7	0.447	0.714	0.021
Log(Root bud no.)	8	0.401	-0.217	-0.741
Log(Main root cardenolides)	9	-0.362	0.278	0.281
Log(Main root C:N)	10	0.742	0.066	0.407
Main root dry mass	11	0.621	0.721	-0.094
Log(Fine root dry mass)	12	0.867	0.010	0.098

Table S3.2 Statistics and loadings from a principal component analysis of 12 growth, defense, and ecophysiological traits of common milkweed *Asclepias syriaca. Notes:* Variable codes correspond to those shown in Figure S1B. Length of the longest leaf and of the main root were not included because we did not detect variation at the population or family level for these two traits (see Table 1). The horizontal line in the middle of the table divides aboveground from belowground plant traits. Abbreviations as in Table 1.

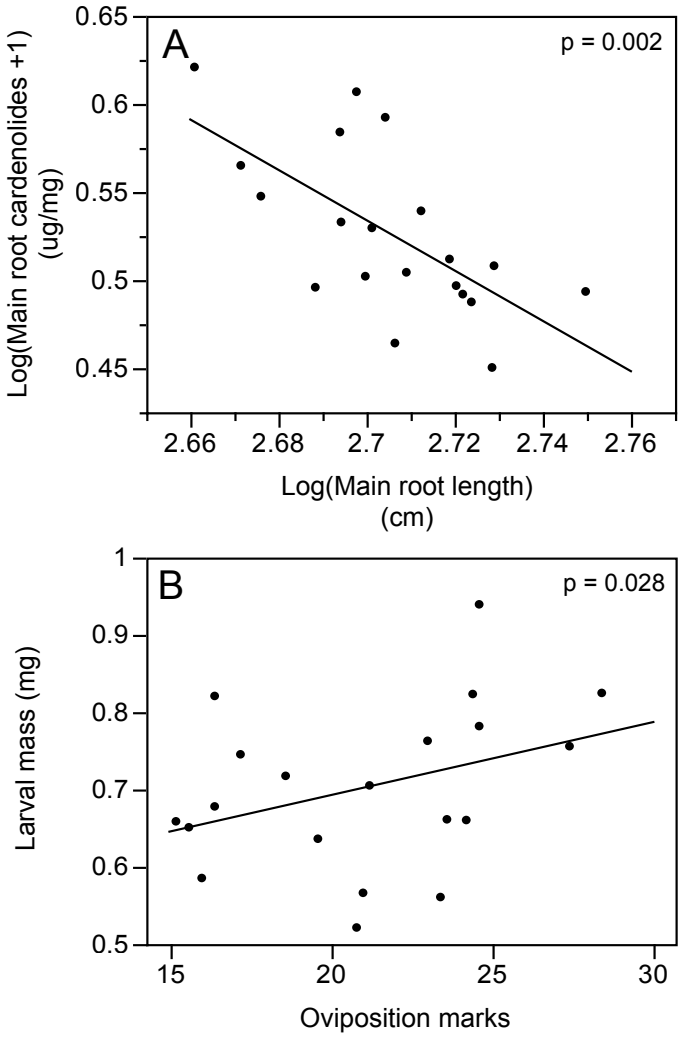

Figure S3.1 Correlations are based on 20 genotypic means, with each dot representing five individual plants grown in a common garden. (A) Correlation between the length and total concentration of cardenolides in *A. syriaca* main roots (as in Table 3.2). (B) Correlation between the number of holes created in oviposition substrate (= grass stems) by individual *T. tetraophthalmus* adult females during a no-choice assay and the mass of individual larvae that were added to root systems of replicate plants of the same genotype on which females fed (as in Table 3.3).

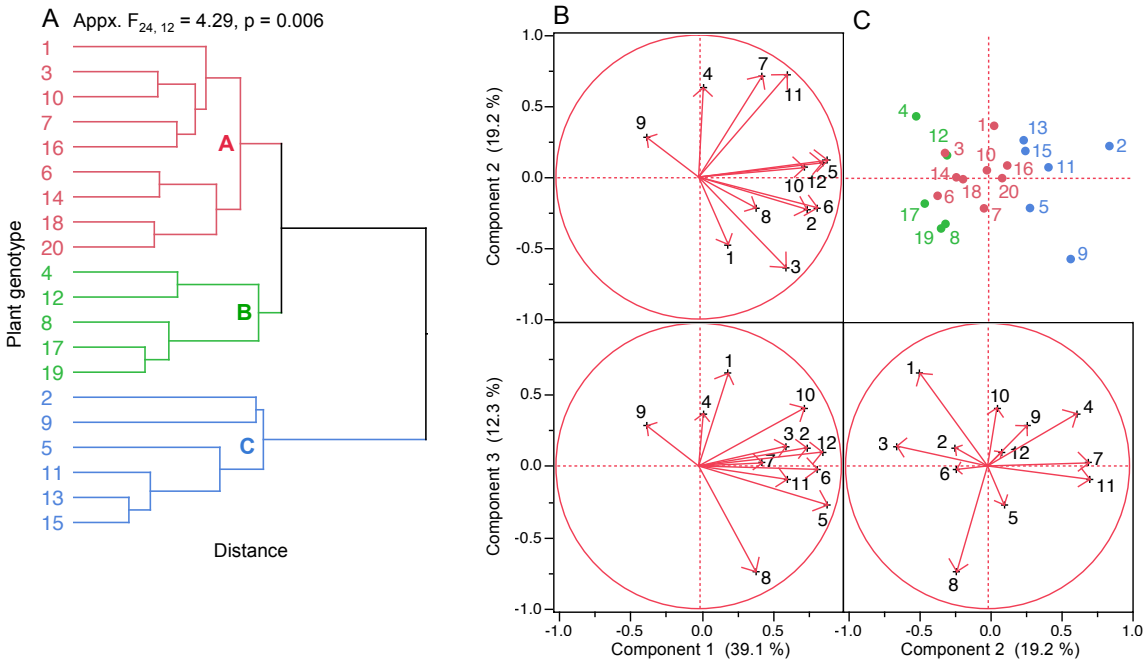

Figure S3.2 A dendrogram (A) that depicts similarity among 20 genotypes of common milkweed *Asclepias syriaca.* The dendrogram was generated by hierarchical cluster analysis of family-level least squared means of 12 above- and belowground traits. Length of the longest leaf and of the main root were not included because we did not detect variation at the population or family level for these two traits (see Table 3.1). Branches were constructed using Ward's minimum variance method and branch spacing is proportional to distance. Tightly clustered families are similar due to phenotype and/or source population (see *Methods*) and can be considered to form potential syndromes, A – C, shown in different colors, in boldface type. Statistical support for the clusters are shown by the approximate F and p values generated from a MANOVA using the Wilks' Lambda method. Due to space limitations, we have coded traits as follows: 1: trichome density; 2: stem height; 3: leaf number; 4: aboveground cardenolide concentration; 5: aboveground C:N ratio; 6: log(aboveground dry mass); 7: main root diameter; 8: log(root bud number); 9: log(main root cardenolide concentration + 1); 10: log(main root C:N ratio); 11: main root dry mass; 12: log(fine root dry mass). (B) Loading plots from a principal component analysis (PCA) of the same 12 traits. Only components with eigenvalues over 1.0 were retained. The red color does not refer to cluster A in panel (A). (C) Score plot from the same PCA showing Component 1 on the x-axis and Component 2 on the y-axis. Colors and numbers correspond to the clusters and genotypes shown in (A) and are not the same as those shown in (B), which refer to traits.

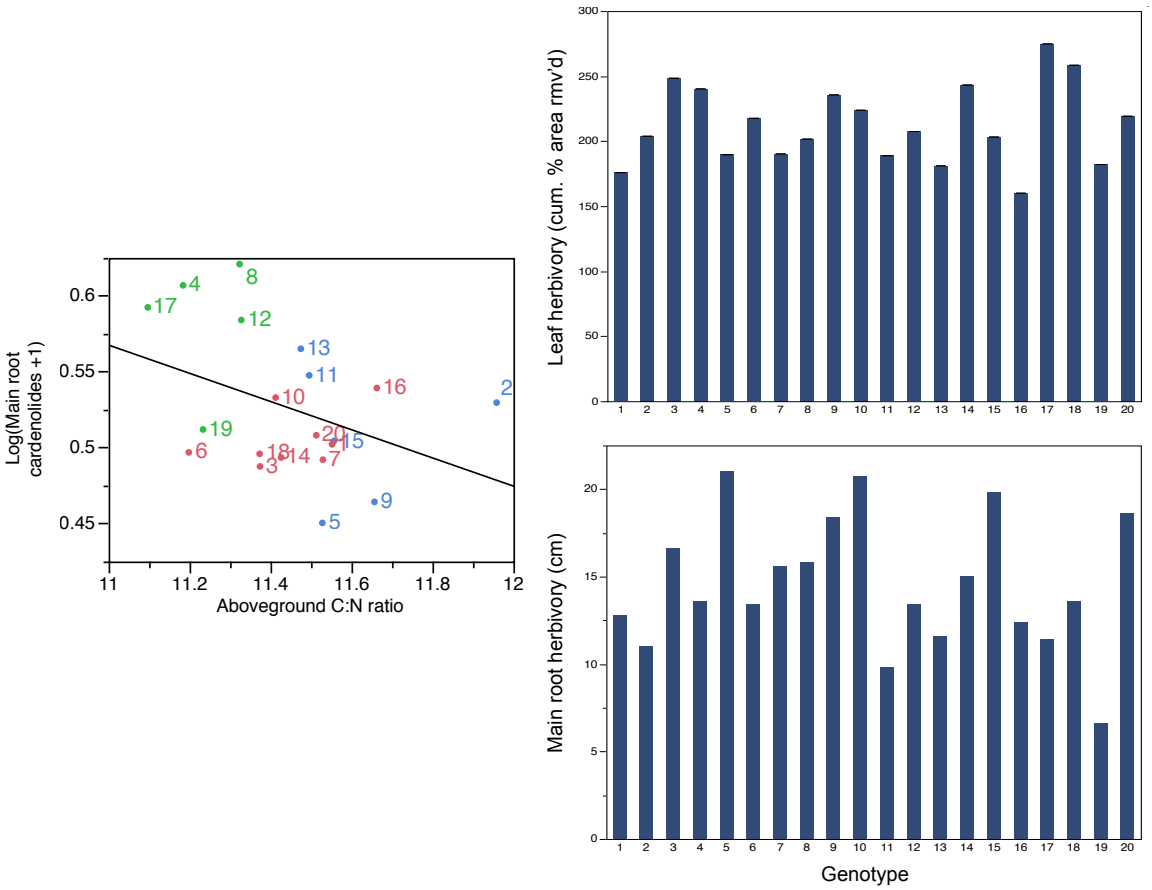

Figure S3.3 Shown are 20 genotypic means, with each dot or bar representing five individual plants grown in a common garden. (A) Correlation between the aboveground C:N ratio and total concentration of cardenolides in *A. syriaca* main roots (as in Table 3.2). Bar graphs of (B) leaf herbivory on, and (C) main root total cardenolide concentration of *A. syriaca* genotypes grown in a common environment (n = 5 plants per genotype).

ALEXIS CLARE ERWIN

EDUCATION

Ph.D. in Ecology and Evolutionary Biology *expected* May 2013
Cornell University, Ithaca, NY
Dissertation: Patterns and ecological consequences of above- and belowground herbivory in common milkweed (*Asclepias syriaca*).
Advisor: Dr. Anurag A. Agrawal.

B.S. *with honors* in Geological Sciences-Biology Dec. 2005
Brown University, Providence, RI
Thesis: Contrasting flower longevity, nectar secretion, and pollination success in two species with convergent pollination syndromes.
Advisor: Dr. Douglass H. Morse.

AWARDS & FELLOWSHIIPS

- Ecological Sociaety of America Graduate Student Policy Award — Mar. 2010
- NSF Graduate Research Fellowship - $30,000 per year for 3 years — Aug. 2007 - Aug. 2010
- Cornell University Olin Fellowship - $23,000 per year for 1 year — Aug. 2006 - Aug. 2007

GRANTS & FUNDING

- Cornell University Summer Research Support — 2012
- NSF Program in Environmental Biocomplexity Research Grants — 2007, 2011
- Cornell University Graduate Student Travel Grants — 2007, 2008, 2010
- Orenstein Endowment Grant — 2009
- Andrew W. Mellon Foundation Graduate Research Award — 2007
- Brown University Undergraduate Teaching and Research Grant — 2005

SCIENCE POLICY EXPERIENCE

Student Liaison, Ecological Society of America - Policy Section Aug. 2012 - present
- Initiated and coordinated a nationwide symposium on policy-relevant environmental science
- Connecting 600 graduate student members to the Society's efforts to advance evidence-based policy

Invited Participant, Biological Sciences Coalition Congressional Visit Apr. 2010
- Wrote a position paper on the impacts of federal funding for basic science research
- Lobbied members of Congress to support increased funding for NSF, USDA, and NOAA

Participant, Environmental Policy Processes course, Cornell in Washington Spring 2009
- Studied the creation, implementation, and evaluation of environmental public policy
- Conducted independent research on the federal budget and appropriations process
- Presented findings in a 20-page policy analysis report

Co-author, Cayuga Lake Working Group, Tompkins Co., NY Jan. 2008 - Dec. 2009
- Formulated indicators to monitor the socio-economic and ecological health of the lake
- Assisted in writing the final report, *A Dashboard for Cayuga Lake*

LEADERSHIP EXPERIENCE

Co-founder, Co-chair, ProDevo, a Professional Development Committee Mar. 2011 - present

- Identified career development needs of 50 graduate students
- Secured funding and institutional support to host a symposium on non-academic careers
- Implementing programs in coordination with the Graduate School and Department

Co-founder, Coordinator, Enviro-Mentors Sep. 2010 - May 2012

- Initiated and facilitate 25 1-on-1 mentoring relationships between graduate students and undergraduates interested in environmental sciences

EEB Representative, Graduate & Professional Student Assembly Aug. 2006 - Aug. 2007

- Represented the interests of 50 Masters and Ph.D. students to the governing body
- Lobbying resulted in a streamlined budget submission and review process

COMMUNICATION EXPERIENCE & TRAINING

Invited Speaker

- Investing for the Future: Federal Science Funding and its Benefits Oct. 2010
  The Ecologist Goes to Washington, Ecological Society of America podcast
- Understanding Nature Through Geological and Biological Science
  Millhauser Symposium in STEM, Park School, Baltimore, MD Apr. 2009

Invited Panelist

- Science Out of Context, Program in Environmental Biocomplexity meeting Nov. 2012
- Possibilities and Perils of Public Engagement, Cornell roundtable event Feb. 2012
- Environmental Policymaking, Ecological Society of America annual meeting Aug. 2010

Training in Science Communication

- Science Communication, COMM 5660, Cornell University Spring 2010
- Communicating Science: Tools for Scientists and Engineers, NSF workshop Nov. 2009
- Public Speaking, NSF-sponsored workshop Sept. 2009
- Interdisciplinary Collaboration, NSF-sponsored workshop Oct. 2008

RESEARCH EXPERIENCE

Graduate Researcher, Cornell University, Ithaca, NY Aug. 2006 - present

- Designed, funded, and conducted a research program in plant ecology
- Independent and collaborative projects will result in six peer-reviewed publications

Research Assistant, UMASS-Dartmouth, New Bedford, MA Jun. - Aug. 2004

- Administered a 19 minute protocol accurately and consistently
- Results were published in a peer-reviewed article about the development of the Southern Ocean

Research Intern, Marine Biological Laboratory, Woods Hole, MA Apr. - Jun. 2001

- Independently developed experimental materials, conducted behavioral trials with colleagues
- Results were published in a peer-reviewed article about chemical signaling between marine animals

TEACHING EXPERIENCE

- Plant Behavior Lecture and Laboratory, BioNB 4460 and 4461 — Spring 2013
- Current Topics in Ecology and Evolutionary Biology, BioEE 7670 — Fall 2012
- Evolutionary Biology and Diversity, BioEE 1780 — Spring 2011
- Fossil Record, Geol 31 — Fall 2004

OUTREACH & MENTORING

Workshop Designer and Leader — Apr. 2009, Apr. 2010
Expanding Your Horizons program, Ithaca, NY

- Designed and led ecology workshops for 120 middle school students from across New York State

Outreach Specialist — May - Jun. 2009
ScienCenter Museum, Ithaca, NY

- Led hands-on science activities for 100 students in grades K through 6
- Assessed the impacts of activities on participants, which facilitated program growth and development

Mentored undergraduates in the following majors: — Summer 2010 - 2012
International Agriculture and Rural Development, Geography, Ecology and Evolutionary Biology

Provided experiential learning opportunities — Summer 2007
Community and Career Apprenticeship Program, The Learning Web, Inc.

PUBLICATIONS

- **A. C. Erwin**, M. A. Geber, A. A. Agrawal. Specific impacts of two root herbivores and soil nutrients on plant performance and insect-insect interactions. *Oikos*. — 2013

- R. Rasmann, **A. C. Erwin**, R. Halitschke, A. A. Agrawal. Direct and indirect root defense of milkweed (*Asclepias syriaca*): trophic cascades, tradeoffs, and novel methods for studying subterranean herbivory. *Journal of Ecology 99: 16-25*. — 2011

- R. Rasmann, A. A. Agrawal, S. C. Cook, **A. C. Erwin**. Cardenolides, induced responses, and interactions between above- and belowground herbivores of milkweeds (*Asclepias* spp.). *Ecology* 90: 2393-2404. — 2009

- A. A. Agrawal, **A. C. Erwin**, S. C. Cook. Natural selection on and predicted response of ecophysiological traits of swamp milkweed (*Asclepias incarnata*). *Journal of Ecology* 96: 536-542. — 2008

- K. C. Buresch, J. G. Boal, J. Knowles, J. Debose, A. Nichols, **A. C. Erwin**, S. D. Painter, G. T. Nagle, R. T. Hanlon. Contact chemosensory cues in egg bundles elicit male–male agonistic conflicts in the squid *Loligo pealeii*. *Journal of Chemical Ecology* 29: 547-560 — 2003

Manuscript referee

- American Journal of Botany
- Botany
- Evolutionary Ecology Research
- Journal of the Kansas Entomological Society
- New Phytologist
- Physiological Entomology

Grant referee

- NSF Program in Environmental Biocomplexity 2007, 2009, 2011
- Orenstein Endowment 2011 - present

Memberships and Affiliations

- Ecological Society of America 2006-present
- Ecological Society of America Policy Section 2011-present
- American Association for the Advancement of Science 2010-present
- NSF Program in Environmental Biocomplexity 2007-2011